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(71) Applicant *(for all designated States except US)*: GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080–4990 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BOTSTEIN, David [US/US]; 2539 Somerset Drive, Belmont, CA 94002 (US). GODDARD, Audrey [CA/US]; 110 Congo Street, San Francisco, CA 94131 (US). GURNEY, Austin [US/US]; 1 Debbie Lane, Belmont, CA 94002 (US). HILLAN, Kenneth [GB/US]; 64 Seward, San Francisco, CA 94114 (US). LAWRENCE, David, A. [US/US]; 1659 12th Avenue, San Francisco, CA 94122 (US). ROY, Margaret [US/US]; 2960 Webster Street #4, San Francisco, CA 94123 (US). WOOD, William, I. [US/US]; 1900 Tarrytown Street, San Mateo, CA 94402 (US).

(74) Agents: DREGER, Ginger et al.; Genentech, Inc, 1 DNA Way, South San Francisco, CA 94080–4990 (US).

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(57) Abstract

The invention concerns compositions and methods for the diagnosis and treatment of neoplastic cell growth and proliferation in mammals, including humans. The invention is based on the identification of genes that are amplified in the genome of tumor cells. Such gene amplification is expected to be associated with the overexpression of the gene product and contribute to tumorigenesis. According, the proteins encoded by the amplified genes are believed to be useful targets for the diagnosis and/or treatment (including prevention) of certain cancers, and may act of predictors of the prognosis of tumor treatment.

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GENES AMPLIFIED IN TUMOURS, ANTIBODIES AGAINST THE PROTEINS ENCODED THEREBY, AND THEIR USE IN DIAGNOSIS AND TREATMENT OF CANCER

Field of the Invention

The present invention relates to compositions and methods for the diagnosis and treatment of tumor.

Background of the Invention

Malignant tumors (cancers) are the second leading cause of death in the United States, after heart disease (Boring et al., CA Cancel J. Clin. 43, 7,[1993]).

Cancer is characterized by the increase in the number of abnormal, or neoplastic, cells derived from a normal tissue which proliferate to form a tumor mass, the invasion of adjacent tissues by these neoplastic tumor cells, and the generation of malignant cells which eventually spread via the blood or lymphatic system to regional lymph nodes and to distant sites (metastasis). In a cancerous state a cell proliferates under conditions in which normal cells would not grow. Cancer manifests itself in a wide variety of forms, characterized by different degrees of invasiveness and aggressiveness.

Alteration of gene expression is intimately related to the uncontrolled cell growth and de-differentiation which are a common feature of all cancers. The genomes of certain well studied tumors have been found to show decreased expression of recessive genes, usually referred to as tumor suppression genes, which would normally function to prevent malignant cell growth, and/or overexpression of certain dominant genes, such as oncogenes, that act to promote malignant growth. Each of these genetic changes appears to be responsible for importing some of the traits that, in aggregate, represent the full neoplastic phenotype (Hunter, Cell 64, 1129 [1991]; Bishop, Cell 64, 235-248 [1991]).

A well known mechanism of gene (e.g. oncogene) overexpression in cancer cells is gene amplification. This is a process where in the chromosome of the ancestral cell multiple copies of a particular gene are produced. The process involves unscheduled replication of the region of chromosome comprising the gene, followed by recombination of the replicated segments back into the chromosome (Alitalo *et al.*, Adv. Cancer Res. 47, 235-281 [1986]). It is believed that the overexpression of the gene parallels gene amplification, i.e. is proportionate to the number of copies made.

Proto-oncogenes that encode growth factors and growth factor receptors have been identified to play important roles in the pathogenesis of various human malignancies, including breast cancer. For example, it has been found that the human ErbB2 gene (*erb*B2, also known as *her2*, or *c-erbB-2*), which encodes a 185-kd transmembrane glycoprotein receptor ($p185^{HER2}$; HER2) related to the epidermal growth factor receptor (EGFR), is overexpressed in about 25% to 30% of human breast cancer (Slamon *et al.*, *Science* 235:177-182 [1987]; Slamon *et al.*, *Science* 244:707-712 [1989]).

It has been reported that gene amplification of a protooncogen is an event typically involved in the more malignant forms of cancer, and could act as a predictor of clinical outcome (Schwab *et al.*, Genes Chromosomes Cancer 1, 181-193 [1990]; Alitalo *et al.*, *supra*). Thus, *erb*B2 overexpression is commonly regarded as a predictor of a poor prognosis, especially in patients with primary disease that involves axillary lymph nodes (Slamon *et al.*, [1987] and [1989], *supra*; Ravdin and Chamness, *Gene* 159:19-27 [1995]; and Hynes and Stern, *Biochim Biophys Acta* 1198:165-184 [1994]), and has been linked to sensitivity and/or resistance to hormone therapy and chemotherapeutic regimens, including CMF (cyclophosphamide, methotrexate, and fluoruracil) and anthracyclines (Baselga *et al.*, *Oncology* 11(3 Suppl 1):43-48 [1997]). However, despite the association of

*erb*B2 overexpression with poor prognosis, the odds of HER2-positive patients responding clinically to treatment with taxanes were greater than three times those of HER2-negative patients (*Ibid*). A recombinant humanized anti-ErbB2 (anti-HER2) monoclonal antibody (a humanized version of the murine anti-ErbB2 antibody 4D5, referred to as rhuMAb HER2 or Herceptin®) has been clinically active in patients with ErbB2-overexpressing metastatic breast cancers that had received extensive prior anticancer therapy. (Baselga *et al.*, *J. Clin. Oncol.* 14:737-744 [1996]).

Summary of the Invention

The present invention concerns compositions and methods for the diagnosis and treatment of neoplastic cell growth and proliferation in mammals, including humans. The present invention is based on the identification of genes that are amplified in the genome of tumor cells. Such gene amplification is expected to be associated with the overexpression of the gene product and contribute to tumorigenesis. Accordingly, the proteins encoded by the amplified genes are believed to be useful targets for the diagnosis and/or treatment (including prevention) of certain cancers, and may act of predictors of the prognosis of tumor treatment.

In one embodiment, the present invention concerns an isolated antibody which binds a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide. In one aspect, the antibody induces death of a cell overexpressing a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide. In another aspect, the antibody is a monoclonal antibody, which preferably has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues. The antibody may be labeled and may be immobilized on a solid support. In a further aspect, the antibody is an antibody fragment, a single-chain antibody, or an anti-idiotypic antibody.

In another embodiment, the invention concerns a composition comprising an antibody which binds a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide in admixture with a pharmaceutically acceptable carrier. In one aspect, the composition comprises a therapeutically effective amount of the antibody. In another aspect, the composition comprises a further active ingredient, which may, for example, be a further antibody or a cytotoxic or chemotherapeutic agent. Preferably, the composition is sterile.

In a further embodiment, the invention concerns nucleic acid encoding an anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody, and vectors and recombinant host cells comprising such nucleic acid.

In a still further embodiment, the invention concerns a method for producing an anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody by culturing a host cell transformed with nucleic acid encoding the antibody under conditions such that the antibody is expressed, and recovering the antibody from the cell culture.

The invention further concerns antagonists and agonists of a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide that inhibit one or more of the functions or activities of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide.

In a further embodiment, the invention concerns isolated nucleic acid molecules that hybridize to the complement of the nucleic acid molecules encoding the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptides. The nucleic acid preferably is DNA, and hybridization

preferably occurs under stringent conditions. Such nucleic acid molecules can act as antisense molecules of the amplified genes identified herein, which, in turn, can find use in the modulation of the respective amplified genes, or as antisense primers in amplification reactions. Furthermore, such sequences can be used as part of ribozyme and/or triple helix sequence which, in turn, may be used in regulation of the amplified genes.

In another embodiment, the invention concerns a method for determining the presence of a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide comprising exposing a cell suspected of containing the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide to an anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody and determining binding of the antibody to the cell.

In yet another embodiment, the present invention concerns a method of diagnosing tumor in a mammal, comprising detecting the level of expression of a gene encoding a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher expression level in the test sample indicates the presence of tumor in the mammal from which the test tissue cells were obtained.

In another embodiment, the present invention concerns a method of diagnosing tumor in a mammal, comprising (a) contacting an anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody with a test sample of tissue cells obtained from the mammal , and (b) detecting the formation of a complex between the anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody and the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide in the test sample. The detection may be qualitative or quantitative, and may be performed in comparison with monitoring the complex formation in a control sample of known normal tissue cells of the same cell type. A larger quantity of complexes formed in the test sample indicates the presence of tumor in the mammal from which the test tissue cells were obtained. The antibody preferably carries a detectable label. Complex formation can be monitored, for example, by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art.

The test sample is usually obtained from an individual suspected to have neoplastic cell growth or proliferation (e.g. cancerous cells).

In another embodiment, the present invention concerns a cancer diagnostic kit, comprising an anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody and a carrier (e.g. a buffer) in suitable packaging. The kit preferably contains instructions for using the antibody to detect the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide.

In yet another embodiment, the invention concerns a method for inhibiting the growth of tumor cells comprising exposing a cell which overexpresses a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide to an effective amount of an agent inhibiting the expression and/or activity of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide. The agent preferably is an anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody, a small organic and inorganic molecule, peptide, phosphopeptide, antisense or ribozyme molecule, or a triple helix molecule. In a specific aspect, the agent, e.g. anti-PRO187,

PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody induces cell death. In a further aspect, the tumor cells are further exposed to radiation treatment and/or a cytotoxic or chemotherapeutic agent.

In a further embodiment, the invention concerns an article of manufacture, comprising:

a container;

a label on the container; and

a composition comprising an active agent contained within the container; wherein the composition is effective for inhibiting the growth of tumor cells, the label on the container indicates that the composition can be used for treating conditions characterized by overexpression of a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide, and the active agent in the composition is an agent inhibiting the expression and/or activity of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide. In a preferred aspect, the active agent is an anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody.

A method for identifying a compound capable of inhibiting the expression and/or activity of a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide, comprising contacting a candidate compound with a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide under conditions and for a time sufficient to allow these two components to interact. In a specific aspect, either the candidate compound or the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide is immobilized on a solid support. In another aspect, the non-immobilized component carries a detectable label.

Brief Description of the Drawings

Figure 1 (SEQ ID NO: 1) shows the nucleotide sequence of DNA27864 that is a cDNA encoding a native sequence FGF-8 homologue (PRO187).

Figure 2 (SEQ ID NO: 2) shows the derived amino acid sequence of a native sequence FGF-8 homologue (PRO187).

Figure 3 describes the Blast score, match and percent homology alignment between the sequence of DNA26645 (SEQ ID NO: 3; a virtual sequence used in the isolation of DNA27864) and the GenBank sequence S78462S3 (SEQ ID NO: 4).

Figure 4 demonstrates the specific binding of PRO533 (UNQ334) encoded by DNA49435 to FGF Receptor 3 (FGFR3).

Figure 5 shows the single stranded nucleotide sequence of a native sequence DNA49435 cDNA (SEQ ID NO: 5).

Figure 6 shows the derived amino acid sequence of a native sequence PRO533 FGF homologue polypeptide (SEQ ID NO: 6).

Figure 7 describes the Blast score, match and percent homology alignment between amino acid residues 3 to 216 of PRO533 (SEQ ID NO: 6) encoded by DNA49435 with residues 6 to 218 of AF007268_1, a fibroblast growth factor sequence (SEQ ID NO: 7).

Figure 8 shows AF007268, an FGF-15 EST sequence (SEQ ID NO: 8) which was used to search various public EST databases (e.g., GenBank, Dayhoff, etc.).

Figure 9 shows the nucleotide sequence of a native sequence EGF-like homologue cDNA DNA32286 (SEQ ID NO: 9).

Figure 10 shows the derived amino acid sequence of a native sequence EGF-like homologue (PRO214; SEQ ID NO: 10) as encoded by the coding sequence of Fig. 9.

Figure 11 shows the nucleotide sequence (SEQ ID NO: 11) of a native sequence PRO240 cDNA, wherein the nucleotide sequence is designated herein as "UNQ214" and/or "DNA34387".

Figure 12 shows the amino acid sequence (PRO240; SEQ ID NO: 12) derived from the nucleotide sequence shown in Figure 11.

Figure 13 shows the consensus nucleotide sequence designated "DNA30873" (SEQ ID NO: 13) derived from a variety of expressed sequence tags (EST's).

Figure 14 shows a BLAST sequence alignment analysis of a portion of the PRO240 amino acid sequence derived from the DNA34387 molecule ("DNA34387") (SEQ ID NO: 14) with the serrate protein precursor from *Drosophilia melanogaster* ("SERR_DROME) (SEQ ID NO: 15).

Figure 15 shows a BLAST sequence alignment analysis of a portion of the PRO240 amino acid sequence derived from the DNA34387 molecule ("DNA34387") (SEQ ID NO: 16) with the C-serrate-1 protein of Gallus gallus ("GGCSERRAT_1") (SEQ ID NO: 17).

Figure 16 shows the nucleotide sequence (SEQ ID NO: 18) of a native sequence EGF-like homologue (designated "DNA32292").

Figure 17 shows the amino acid sequence (PRO211; SEQ ID NO: 19) derived from the nucleotide sequence shown in Figure 16.

Figure 18 shows the amino acid sequence (SEQ ID NO: 20) of PRO230 derived from the nucleotide sequence "DNA33223".

Figure 19 shows the consensus nucleotide sequence "DNA30857" (SEQ ID NO: 21) derived from the alignment of nucleotide sequence from a variety of expressed sequence tags (ESTs).

Figure 20 shows the nucleotide sequence (SEQ ID NO: 22) of a native sequence PRO230 cDNA, wherein the nucleotide sequence is designated herein as "DNA33223". As used herein, "DNA33223" is used interchangeably with "UNQ204".

Figure 21 shows an amino acid sequence alignment of PRO230 (residues 44-463; SEQ ID NO: 23) with a portion of a rabbit tubulointerstitial nephritis antigen precursor (SEQ ID NO: 24).

Figure 22 shows a nucleotide sequence (SEQ ID NO: 25) containing the nucleotide sequence (SEQ ID NO: 26, beginning with nucleotide 10 of SEQ ID NO: 25) of a native sequence PRO261 cDNA, wherein the nucleotide sequence (SEQ ID NO: 25) is a clone, designated as "UNQ228" and/or "DNA33473-se min". Also presented is the position of the initiator methionine residue, circled, at positions 1-3 of SEQ ID NO: 26.

Figure 23 shows the amino acid sequence (PRO261; SEQ ID NO: 27) derived from SEQ ID NO: 26.

Figure 24 shows a consensus nucleotide sequence designated "DNA308430 from dna" (SEQ ID No: 28) derived from the alignment of a number of EST sequences.

Figure 25 shows a BLAST alignment of a portion of the PRO261 amino acid sequence derived from the DNA33473-seq min molecule (SEQ ID No: 29) with a portion of human CTGF (SEQ ID NO: 30).

Figure 26 shows a nucleotide sequence (SEQ ID No: 31) containing the nucleotide sequence (SEQ ID NO: 32) of a native sequence PRO246 cDNA (nucleotides 126-1295), wherein the nucleotide sequence (SEQ ID No: 31) is designated herein as "UNQ220" and/or "DNA35639". The putative transmembrane domain of the protein is encoded by nucleotides beginning at nucleotide 855 in the figure.

Figure 27 shows the amino acid sequence (PRO 246; SEQ ID NO: 33) derived from nucleotides 126-1295 of the nucleotide sequence shown in Figure 26.

Figure 28 shows a consensus nucleotide sequence designated "DNA30955" (SEQ ID NO: 34) derived from the alignment of various EST sequences.

Figure 29 shows a BLAST sequence alignment of a portion of the PRO246 amino acid sequence derived from the DNA35639 molecule ("DNA35639") (SEQ ID NO: 35) with a portion of the human cell surface protein HCAR ("HSU907161_1", SEQ ID NO: 36).

Figure 30 shows the nucleotide sequence of a native-sequence EBAF-2 cDNA (SEQ ID NO: 40), its complementary sequence (SEQ ID NO: 38), the nucleotide sequence encoding residues 1-366 of the deduced amino acid sequence of a native-sequence EBAF-2 (SEQ ID NO: 39), and the derived amino acid sequence of a native-sequence EBAF-2 (SEQ ID NO: 40).

Figure 31 shows the virtual DNA 28722 (SEQ ID NO: 41) from which the oligonucleotide PCR primers were generated to screen various libraries to isolate a full-length sequence.

Figure 32 describes the BLAST^{TM} score, match, and percent homology alignment between the protein human EBAF-2 (SEQ ID NO: 40), encoded by DNA33461 (SEQ ID NO: 37), and human EBAF (hereafter called EBAF-1) (SEQ ID NO: 42).

Figure 33 is a family tree showing the relationship between the EBAF-2 protein and other members of the TGF-β superfamily.

Figure 34 is an *in situ* image showing the expression of DNA49435 over the gallbladder epithelium in adult tissues.

Figure 35 is an *in situ* image showing moderate expression of DNA32286 in placental stromal cells in membranous tissues.

Figure 36 is an *in situ* image showing moderate expression of DNA32286 in thyroid.

Figure 37 is an *in situ* image showing low level expression of DNA32286 in cortical neurons.

Figure 38 is an *in situ* image showing an intense signal associated with the expression of DNA33223 in arterial and venous vessels in the fetus. In arteries, the signal appeared to be confined to smooth-muscle/pericytic cells.

Figure 39 is an *in situ* image showing strong expression of DNA33223 in cells within placental trophoblastic villi.

Figure 40 in an *in situ* image showing strong expression of DNA35639 in fetal vascular endothelium.

Figure 41 shows the consensus sequence DNA28744 used in the identification of cDNA (DNA32286) encoding PRO214 (an EGF-like homologue).

Figure 42 shows the results of framework and epicenter mapping of PRO230 .

Figure 43 shows the results of epicenter and framework mapping of PR0240.

Detailed Description of the Invention

I. Definitions

The phrases "gene amplification" and "gene duplication" are used interchangeably and refer to a process by which multiple copies of a gene or gene fragment are formed in a particular cell or cell line. The duplicated region (a stretch of amplified DNA) is often referred to as "amplicon." Usually, the amount of the messenger RNA (mRNA) produced, i.e. the level of gene expression, also increases in the proportion of the number of copies made of the particular gene expressed.

"Tumor", as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include breast cancer, prostate cancer, colon cancer, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer.

"Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. In tumor (e.g. cancer) treatment, a therapeutic agent may directly decrease the pathology of tumor cells, or render the tumor cells more susceptible to treatment by other therapeutic agents, e.g. radiation and/or chemotherapy.

The "pathology" of cancer includes all phenomena that compromise the well-being of the patient. This includes, without limitation, abnormal or uncontrollable cell growth, metastasis, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels, suppression or aggravation of inflammatory or immunological response, etc.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, *etc.* Preferably, the mammal is human.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.* I^{131}, I^{125}, Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include adriamycin, doxorubicin, epirubicin, 5-fluorouracil, cytosine arabinoside ("Ara-C"), cyclophosphamide, thiotepa, busulfan, cytoxin, taxoids, e.g. paclitaxel (Taxol, Bristol-Myers Squibb Oncology, Princeton, NJ), and doxetaxel (Taxotere, Rhône-Poulenc Rorer, Antony, Rnace), toxotere, methotrexate, cisplatin, melphalan, vinblastine, bleomycin, etoposide, ifosfamide, mitomycin C, mitoxantrone, vincristine, vinorelbine, carboplatin, teniposide, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, esperamicins (see U.S. Pat. No. 4,675,187), melphalan and other related nitrogen mustards. Also included in this definition are hormonal agents that act to regulate or inhibit hormone action on tumors such as tamoxifen and onapristone.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially cancer cell overexpressing any of the genes identified herein, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent is one which significantly reduces the percentage of cells overexpressing such genes in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxol, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogens, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13.

"Doxorubicin" is an athracycline antibiotic. The full chemical name of doxorubicin is (8S-cis)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexapyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione.

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-

CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

As used herein, the terms a "PRO187" polypeptide ("UNQ161", an FGF-8 homologue); a "PRO533" polypeptide ("UNQ334", an FGF homologue); a "PRO214" polypeptide ("UNQ188", an EGF-like homologue); a "PRO240" polypeptide ("UNQ214"; a serrate homologue); a "PRO211" polypeptide ("UNQ185", an EGF-like homologue); a "PRO230" polypeptide ("UNQ204", a polypeptide having homology to tubulointerstitial nephritis antigen (TIN)); a "PRO261" polypeptide ("UNQ228", a polypeptide having homology to the connective tissue growth factor (CTGF)); a "PRO246" polypeptide ("UNQ220", a polypeptide having homology to the cell surface protein HCAR); and an "EBAF-2" polypeptide (a TGF-β superfamily member) are used to refer to a polypeptide comprising a native sequence polypeptide having the same amino acid sequence as a corresponding PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide derived from nature, and fragments of such native sequence polypeptides. Such native sequence PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 can be isolated from nature or, along with the respective fragments, can be produced by recombinant and/or synthetic means. The term specifically encompasses naturally-occurring truncated or secreted forms (*e.g.*, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide. In one embodiment of the invention, the native sequence PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 is a full-length native presequence or a mature form of a PRO187 polypeptide shown in Figure 2 (SEQ ID NO: 2); PRO533 polypeptide shown in Figure 6 (SEQ ID NO: 6); PRO214 polypeptide shown in Figure 10 (SEQ ID NO: 10); PRO240 polypeptide shown in Figure 12 (SEQ ID NO: 12); PRO211 polypeptide shown in Figure 17 (SEQ ID NO: 19); PRO230 polypeptide shown in Figure 18 (SEQ ID NO: 20); PRO261 polypeptide shown in Figure 23 (SEQ ID NO: 27); PRO246 polypeptide shown in Figure 27 (SEQ ID NO: 33), and EBAF-2 polypeptide shown in Figure 30 (SEQ ID NO: 40), respectively. Fragments of the respective native polypeptides herein include, but are not limited, to polypeptide variants from which the native N-terminal signal sequence has been fully or partially deleted or replaced by another sequence, and extracellular domains of the respective native sequences, regardless whether such truncated (secreted) forms occur in nature.

An "isolated" nucleic acid molecule encoding a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2-encoding nucleic acid. An isolated PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2-encoding nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule encoding a PRO187, PRO533, PRO214, PRO240,

PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide includes nucleic acid molecules contained in cells that ordinarily express PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2, where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel *et al.*, Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and % SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a

solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/mL denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

"Active" or "activity" in the context of molecules identified based upon the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptides (or their coding sequences) refers to polypeptides (e.g. antibodies) or organic or inorganic small molecules, peptides, etc. which retain the biological and/or immunological activities/properties of a native or naturally-occurring PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2.

"Biological activity" in the context of an antibody or another molecule that can be identified by the screening assays disclosed herein (e.g. an organic or inorganic small molecule, peptide, etc.) is used to refer to the ability of such molecules to bind or complex with the polypeptides encoded by the amplified genes identified herein, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. A preferred biological activity is growth inhibition of a target tumor cell. Another preferred biological activity is cytotoxic activity resulting in the death of the target tumor cell.

The phrase "immunological property" means immunological cross-reactivity with at least one epitope of a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide.

"Immunological cross-reactivity" as used herein means that the candidate polypeptide is capable of competitively inhibiting the qualitative biological activity of a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide having this activity with polyclonal antisera raised against the known active PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide. Such antisera are prepared in conventional fashion by injecting goats or rabbits, for example, subcutaneously with the known active analogue in complete Freund's adjuvant, followed by booster intraperitoneal or subcutaneous injection in incomplete Freunds. The immunological cross-reactivity preferably is "specific", which means that the binding affinity of the immunologically cross-reactive molecule (e.g. antibody) identified, to the corresponding PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide is significantly higher (preferably at least about 2-times, more preferably at least about 4-times, even more preferably at least about 8-times, most preferably at least about 8-times higher) than the binding affinity of that molecule to any other known native polypeptide.

The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native PRO187, PRO533, PRO214, PRO240,

PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native polypeptides, peptides, small organic molecules, etc.

A "small molecule" is defined herein to have a molecular weight below about 500 daltons.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. The term "antibody" is used in the broadest sense and specifically covers, without limitation, intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *NIH Publ. No.91-3242*, Vol. I, pages 647-669 (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (Zapata *et al.* , Protein Eng. 8(10):1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ϵ, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, Nature, 256:495 [1975], or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody

libraries using the techniques described in Clackson *et al.*, Nature, 352:624-628 [1991] and Marks *et al.*, J. Mol. Biol., 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al.*, Proc. Natl. Acad. Sci. USA, 81:6851-6855 [1984]).

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, Nature, 321:522-525 (1986); Reichmann *et al.*, Nature, 332:323-329 [1988]; and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992). The humanized antibody includes a PRIMATIZED™antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest.

"Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and

other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (*e.g.* radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (*e.g.*, controlled pore glass), polysaccharides (*e.g.*, agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (*e.g.*, an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as an PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide or an antibody thereto and, optionally, a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

II. Compositions and Methods of the Invention

1. Preparation of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, and EBAF-2 polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO187 (UNQ161), PRO533 (UNQ334), PRO214 (UNQ188), PRO240 (UNQ214), PRO211 (UNQ185), PRO230 (UNQ204), PRO261 (UNQ228), PRO246 (UNQ220), or EBAF-2. In particular, cDNAs encoding certain PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, and EBAF-2 polypeptides have been identified and isolated, as disclosed in further detail in the Examples below. It is noted that proteins produced in separate expression rounds may be given

different PRO numbers but the UNQ number is unique for any given DNA and the encoded protein, and will not be changed. However, for sake of simplicity, in the present specification the proteins encoded by nucleic acid referred to as "DNA27864", "DNA49435", "DNA32286", "DNA34387", "DNA32292", "DNA33223", "DNA33473", "DNA35639", and "DNA33461", as well as all further native homologues and variants included in the foregoing definition of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide, will be referred to as "PRO187", "PRO533", "PRO214", "PRO240", "PRO211", "PRO230", "PRO261", "PRO246", or "EBAF-2" polypeptide, regardless of their origin or mode of expression.

The description below relates primarily to production of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, and EBAF-2 polypeptides by culturing cells transformed or transfected with a vector containing PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2-encoding nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptides. For instance, the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart et al., Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, J. Am. Chem. Soc., 85:2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2.

i. Isolation of DNA Encoding a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide.

DNA encoding PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 may be obtained from a cDNA library prepared from tissue believed to possess the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 mRNA and to express it at a detectable level. Accordingly, human PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. The PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2-encoding gene may also be obtained from a genomic library or by oligonucleotide synthesis.

Libraries can be screened with probes (such as antibodies to the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide, or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 is to use PCR methodology [Sambrook et al., *supra*; Dieffenbach et al., PCR Primer: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like ^{32}P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook et al., *supra*.

Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined through sequence alignment using computer software programs such as ALIGN, DNAstar, and INHERIT which employ various algorithms to measure homology.

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook et al., supra, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

ii. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in Mammalian Cell Biotechnology: a Practical Approach, M. Butler, ed. (IRL Press, 1991) and Sambrook et al., *supra*.

Methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook et al., *supra*, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transformations have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen et al., J. Bact., 130:946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown et al., Methods in Enzymology, 185:527-537 (1990) and Mansour et al., Nature, 336:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains

are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635).

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism.

Suitable host cells for the expression of glycosylated PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as Drosophila S2 and Spodoptera Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol., 36:59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

iii. Selection and Use of a Replicable Vector

The nucleic acid (*e.g.*, cDNA or genomic DNA) encoding PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

The PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub et al., Proc. Natl. Acad. Sci. USA, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp*1 gene present in the yeast plasmid YRp7 [Stinchcomb et al., Nature, 282:39 (1979); Kingsman et al., Gene, 7:141 (1979); Tschemper et al., Gene, 10:157 (1980)]. The *trp*1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, Genetics, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems [Chang et al., Nature, 275:615 (1978); Goeddel et al., Nature, 281:544 (1979)], alkaline phosphatase, a tryptophan (trp) promoter system [Goeddel, Nucleic Acids Res., 8:4057 (1980); EP 36,776], and hybrid promoters such as the tac promoter [deBoer et al., Proc. Natl. Acad. Sci. USA, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman et al., J. Biol. Chem., 255:2073 (1980)] or other glycolytic enzymes [Hess et al., J. Adv. Enzyme Reg., 7:149 (1968); Holland, Biochemistry, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism. metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the

genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 coding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 in recombinant vertebrate cell culture are described in Gething et al., Nature, 293:620-625 (1981); Mantei et al., Nature, 281:40-46 (1979); EP 117,060; and EP 117,058.

iv. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide or against a synthetic peptide based on the DNA sequences provided

herein or against exogenous sequence fused to PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 DNA and encoding a specific antibody epitope.

v. Purification of Polypeptide

Forms of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptides may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.* Triton-X 100) or by enzymatic cleavage. Cells employed in expression of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptides. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, Methods in Enzymology, 182 (1990); Scopes, Protein Purification: Principles and Practice, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide produced.

2. Amplification of Genes Encoding the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 Polypeptides in Tumor Tissues and Cell Lines

The present invention is based on the identification and characterization of genes which are amplified in certain cancer cells.

The genome of prokaryotic and eukaryotic organisms is subjected to two seemingly conflicting requirements. One is the preservation and propagation of DNA as the genetic information in its original form, to guarantee stable inheritance through multiple generations. On the other hand, cells or organisms must be able to adapt to lasting environmental changes. The adaptive mechanisms can include qualitative or quantitative modifications of the genetic material. Qualitative modifications include DNA mutations, in which coding sequences are altered resulting in a structurally and/or functionally different protein. Gene amplification is a quantitative modification, whereby the actual number of complete coding sequence, i.e. a gene, increases, leading to an increased number of available templates for transcription, an increased number of translatable transcripts, and, ultimately, to an increased abundance of the protein encoded by the amplified gene.

The phenomenon of gene amplification and its underlying mechanisms have been investigated *in vitro* in several prokaryotic and eukaryotic culture systems. The best-characterized example of gene amplification involves the culture of eukaryotic cells in medium containing variable concentrations of the cytotoxic drug methotrexate (MTX). MTX is a folic acid analogue and interferes with DNA synthesis by blocking the enzyme dihydrofolate reductase (DHFR). During the initial exposure to low concentrations of MTX

most cells (>99.9%) will die. A small number of cells survive, and are capable of growing in increasing concentrations of MTX by producing large amounts of DHFR-RNA and protein. The basis of this overproduction is the amplification of the single DHFR gene. The additional copies of the gene are found as extrachromosomal copies in the form of small, supernumerary chromosomes (double minutes) or as integrated chromosomal copies.

Gene amplification is most commonly encountered in the development of resistance to cytotoxic drugs (antibiotics for bacteria and chemotherapeutic agents for eukaryotic cells) and neoplastic transformation. Transformation of a eukaryotic cell as a spontaneous event or due to a viral or chemical/environmental insult is typically associated with changes in the genetic material of that cell. One of the most common genetic changes observed in human malignancies are mutations of the p53 protein. p53 controls the transition of cells from the stationary (G1) to the replicative (S) phase and prevents this transition in the presence of DNA damage. In other words, one of the main consequences of disabling p53 mutations is the accumulation and propagation of DNA damage, i.e. genetic changes. Common types of genetic changes in neoplastic cells are, in addition to point mutations, amplifications and gross, structural alterations, such as translocations.

The amplification of DNA sequences may indicate specific functional requirement as illustrated in the DHFR experimental system. Therefore, the amplification of certain oncogenes in malignancies points toward a causative role of these genes in the process of malignant transformation and maintenance of the transformed phenotype. This hypothesis has gained support in recent studies. For example, the *bcl-2* protein was found to be amplified in certain types of non-Hodgkin's lymphoma. This protein inhibits apoptosis and leads to the progressive accumulation of neoplastic cells. Members of the gene family of growth factor receptors have been found to be amplified in various types of cancers suggesting that overexpression of these receptors may make neoplastic cells less susceptible to limiting amounts of available growth factor. Examples include the amplification of the androgen receptor in recurrent prostate cancer during androgen deprivation therapy and the amplification of the growth factor receptor homologue ERB2 in breast cancer. Lastly, genes involved in intracellular signaling and control of cell cycle progression can undergo amplification during malignant transformation. This is illustrated by the amplification of the *bcl-I* and *ras* genes in various epithelial and lymphoid neoplasms.

These earlier studies illustrate the feasibility of identifying amplified DNA sequences in neoplasms, because this approach can identify genes important for malignant transformation. The case of ERB2 also demonstrates the feasibility from a therapeutic standpoint, since transforming proteins may represent novel and specific targets for tumor therapy.

Several different techniques can be used to demonstrate amplified genomic sequences. Classical cytogenetic analysis of chromosome spreads prepared from cancer cells is adequate to identify gross structural alterations, such as translocations, deletions and inversions. Amplified genomic regions can only be visualized, if they involve large regions with high copy numbers or are present as extrachromosomal material. While cytogenetics was the first technique to demonstrate the consistent association of specific chromosomal changes with particular neoplasms, it is inadequate for the identification and isolation of manageable DNA sequences. The more recently developed technique of comparative genomic hybridization (CGH) has illustrated the widespread phenomenon of genomic amplification in neoplasms. Tumor and normal DNA are hybridized

simultaneously onto metaphases of normal cells and the entire genome can be screened by image analysis for DNA sequences that are present in the tumor at an increased frequency. (WO 93/18,186; Gray *et al.*, Radiation Res. 137, 275-289 [1994]). As a screening method, this type of analysis has revealed a large number of recurring amplicons (a stretch of amplified DNA) in a variety of human neoplasms. Although CGH is more sensitive than classical cytogenetic analysis in identifying amplified stretches of DNA, it does not allow a rapid identification and isolation of coding sequences within the amplicon by standard molecular genetic techniques.

The most sensitive methods to detect gene amplification are polymerase chain reaction (PCR)-based assays. These assays utilize very small amount of tumor DNA as starting material, are exquisitely sensitive, provide DNA that is amenable to further analysis, such as sequencing and are suitable for high-volume throughput analysis.

The above-mentioned assays are not mutually exclusive, but are frequently used in combination to identify amplifications in neoplasms. While cytogenetic analysis and CGH represent screening methods to survey the entire genome for amplified regions, PCR-based assays are most suitable for the final identification of coding sequences, i.e. genes in amplified regions.

According to the present invention, such genes have been identified by quantitative PCR (S. Gelmini *et al.*, Clin. Chem. 43, 752 [1997]), by comparing DNA from a variety of primary tumors, including breast, lung, colon, prostate, brain, liver, kidney, pancreas, spleen, thymus, testis, ovary, uterus, etc. tumor, or tumor cell lines, with pooled DNA from healthy donors. Quantitative PCR was performed using a TaqMan instrument (ABI). Gene-specific primers and fluorogenic probes were designed based upon the coding sequences of the DNAs.

Human lung carcinoma cell lines include A549 (SRCC768), Calu-1 (SRCC769), Calu-6 (SRCC770), H157 (SRCC771), H441 (SRCC772), H460 (SRCC773), SKMES-1 (SRCC774) and SW900 (SRCC775), all available from ATCC. Primary human lung tumor cells usually derive from adenocarcinomas, squamous cell carcinomas, large cell carcinomas, non-small cell carcinomas, small cell carcinomas, and broncho alveolar carcinomas, and include, for example, SRCC724 (squamous cell carcinoma abbreviated as "SqCCa"), SRCC725 (non-small cell carcinoma, abbreviated as "NSCCa"), SRCC726 (adenocarcinoma, abbreviated as "AdenoCa"), SRCC727 (adenocarcinoma), SRCC728 (squamous cell carcinoma), SRCC729 (adenocarcinoma), SRCC730 (adeno/squamous cell carcinoma), SRCC731 (adenocarcinoma), SRCC732 (squamous cell carcinoma), SRCC733 (adenocarcinoma), SRCC734 (adenocarcinoma), SRCC735 (broncho alveolar carcinoma, abbreviated as "BAC"), SRCC736 (squamous cell carcinoma), SRCC738 (squamous cell carcinoma), SRCC739 (squamous cell carcinoma), SRCC740 (squamous cell carcinoma), SRCC740 (lung cell carcinoma, abbreviated as "LCCa").

Colon cancer cell lines include, for example, ATCC cell lines SW480 (adenocarcinoma, SRCC776), SW620 (lymph node metastasis of colon adenocarcinoma, SRCC777), COLO320 (adenocarcinoma, SRCC778), HT29 (adenocarcinoma, SRCC779), HM7 (carcinoma, SRCC780), CaWiDr (adenocarcinoma, srcc781), HCT116 (carcinoma, SRCC782), SKCO1 (adenocarcinoma, SRCC783), SW403 (adenocarcinoma, SRCC784), LS174T (carcinoma, SRCC785), and HM7 (a high mucin producing variant of ATCC colon adenocarcinoma cell line LS 174T, obtained from Dr. Robert Warren, UCSF). Primary colon tumors include colon adenoocarcinomas designated CT2 (SRCC742), CT3 (SRCC743), CT8 (SRCC744), CT10 (SRCC745), CT12 (SRCC746), CT14 (SRCC747), CT15 (SRCC748), CT17 (SRCC750), CT1 (SRCC751), CT4 (SRCC752),

CT5 (SRCC753), CT6 (SRCC754), CT7 (SRCC755), CT9 (SRCC756), CT11 (SRCC757), CT18 (SRCC758), and DcR3, BACrev, BACfwd, T160, and T159.

Human breast carcinoma cell lines include, for example, HBL100 (SRCC759), MB435s (SRCC760), T47D (SRCC761), MB468(SRCC762), MB175 (SRCC763), MB361 (SRCC764), BT20 (SRCC765), MCF7 (SRCC766), SKBR3 (SRCC767).

3. Tissue Distribution

The results of the gene amplification assays herein can be verified by further studies, such as, by determining mRNA expression in various human tissues.

As noted before, gene amplification and/or gene expression in various tissues may be measured by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes.

Gene expression in various tissues, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 DNA and encoding a specific antibody epitope. General techniques for generating antibodies, and special protocols for Northern blotting and *in situ* hybridization are provided hereinbelow.

4. Chromosome Mapping

If the amplification of a given gene is functionally relevant, then that gene should be amplified more than neighboring genomic regions which are not important for tumor survival. To test this, the gene can be mapped to a particular chromosome, e.g. by radiation-hybrid analysis. The amplification level is then determined at the location identified, and at neighboring genomic region. Selective or preferential amplification at the genomic region to which to gene has been mapped is consistent with the possibility that the gene amplification observed promotes tumor growth or survival. Chromosome mapping includes both framework and epicenter mapping. For further details see e.g., Stewart *et al.*, Genome Research 7, 422-433 (1997).

5. Antibody Binding Studies

The results of the gene amplification study can be further verified by antibody binding studies, in which the ability of anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibodies to inhibit the effect of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptides on tumor (cancer) cells is tested. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies, the preparation of which will be described hereinbelow.

Antibody binding studies may be carried out in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp.147-158 (CRC Press, Inc., 1987).

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of target protein (encoded by a gene amplified in a tumor cell) in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies preferably are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, *e.g.*, US Pat No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

For immunohistochemistry, the tumor sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.

6. Cell-Based Tumor Assays

Cell-based assays and animal models for tumors (e.g. cancers) can be used to verify the findings of the gene amplification assay, and further understand the relationship between the genes identified herein and the development and pathogenesis of neoplastic cell growth. The role of gene products identified herein in the development and pathology of tumor or cancer can be tested by using primary tumor cells or cells lines that have been identified to amplify the genes herein. Such cells include, for example, the breast, colon and lung cancer cells and cell lines listed above.

In a different approach, cells of a cell type known to be involved in a particular tumor are transfected with the cDNAs herein, and the ability of these cDNAs to induce excessive growth is analyzed. Suitable cells include, for example, stable tumor cells lines such as, the B104-1-1 cell line (stable NIH-3T3 cell line transfected with the *neu* protooncogene) and *ras*-transfected NIH-3T3 cells, which can be transfected with the desired gene, and monitored for tumorogenic growth. Such transfected cell lines can then be used to test the ability of poly- or monoclonal antibodies or antibody compositions to inhibit tumorogenic cell growth by exerting cytostatic or cytotoxic activity on the growth of the transformed cells, or by mediating antibody-dependent cellular cytotoxicity (ADCC). Cells transfected with the coding sequences of the genes identified herein can further be used to identify drug candidates for the treatment of cancer.

In addition, primary cultures derived from tumors in transgenic animals (as described below) can be used in the cell-based assays herein, although stable cell lines are preferred. Techniques to derive continuous cell lines from transgenic animals are well known in the art (see, e.g. Small *et al.*, Mol. Cell. Biol. 5, 642-648 [1985]).

7. Animal Models

A variety of well known animal models can be used to further understand the role of the genes identified herein in the development and pathogenesis of tumors, and to test the efficacy of candidate therapeutic agents, including antibodies, and other antagonists of the native polypeptides, including small molecule antagonists. The *in vivo* nature of such models makes them particularly predictive of responses in human patients. Animal models of tumors and cancers (e.g. breast cancer, colon cancer, prostate cancer, lung cancer, etc.) include both non-recombinant and recombinant (transgenic) animals. Non-recombinant animal models include, for example, rodent, e.g., murine models. Such models can be generated by introducing tumor cells into syngeneic mice using standard techniques, e.g. subcutaneous injection, tail vein injection, spleen implantation, intraperitoneal implantation, implantation under the renal capsule, or orthopin implantation, e.g. colon cancer cells implanted in colonic tissue. (See, e.g. PCT publication No. WO 97/33551, published September 18, 1997).

Probably the most often used animal species in oncological studies are immunodeficient mice and, in particular, nude mice. The observation that the nude mouse with hypo/aplasia could successfully act as a host for human tumor xenografts has lead to its widespread use for this purpose. The autosomal recessive *nu* gene has been introduced into a very large number of distinct congenic strains of nude mouse, including, for example, ASW, A/He, AKR, BALB/c, B10.LP, C17, C3H, C57BL, C57, CBA, DBA, DDD, I/st, NC, NFR, NFS, NFS/N, NZB, NZC, NZW, P, RIII and SJL. In addition, a wide variety of other animals with inherited immunological defects other than the nude mouse have been bred and used as recipients of tumor xenografts. For further details see, e.g. *The Nude Mouse in Oncology Research*, E. Boven and B. Winograd, eds., CRC Press, Inc., 1991.

The cells introduced into such animals can be derived from known tumor/cancer cell lines, such as, any of the above-listed tumor cell lines, and, for example, the B104-1-1 cell line (stable NIH-3T3 cell line transfected with the *neu* protooncogene); *ras*-transfected NIH-3T3 cells; Caco-2 (ATCC HTB-37); a moderately well-differentiated grade II human colon adenocarcinoma cell line, HT-29 (ATCC HTB-38), or from tumors and cancers. Samples of tumor or cancer cells can be obtained from patients undergoing surgery, using standard conditions, involving freezing and storing in liquid nitrogen (Karmali *et al.*, Br. J. Cancer 48, 689-696 [1983]).

Tumor cells can be introduced into animals, such as nude mice, by a variety of procedures. The subcutaneous (s.c.) space in mice is very suitable for tumor implantation. Tumors can be transplanted s.c. as solid blocks, as needle biopsies by use of a trochar, or as cell suspensions. For solid block or trochar implantation, tumor tissue fragments of suitable size are introduced into the s.c. space. Cell suspensions are freshly prepared from primary tumors or stable tumor cell lines, and injected subcutaneously. Tumor cells can also be injected as subdermal implants. In this location, the inoculum is deposited between the lower part of the dermal connective tissue and the s.c. tissue. Boven and Winograd (1991), *supra*.

Animal models of breast cancer can be generated, for example, by implanting rat neuroblastoma cells (from which the *neu* oncogen was initially isolated), or *neu* -transformed NIH-3T3 cells into nude mice, essentially as described by Drebin *et al.* PNAS USA 83, 9129-9133 (1986).

Similarly, animal models of colon cancer can be generated by passaging colon cancer cells in animals, e.g. nude mice, leading to the appearance of tumors in these animals. An orthotopic transplant model of human colon cancer in nude mice has been described, for example, by Wang *et al.*, Cancer Research 54, 4726-

4728 (1994) and Too *et al.*, Cancer Research 55, 681-684 (1995). This model is based on the so-called "METAMOUSE" sold by AntiCancer, Inc. (San Diego, California).

Tumors that arise in animals can be removed and cultured *in vitro*. Cells from the *in vitro* cultures can then be passaged to animals. Such tumors can serve as targets for further testing or drug screening. Alternatively, the tumors resulting from the passage can be isolated and RNA from pre-passage cells and cells isolated after one or more rounds of passage analyzed for differential expression of genes of interest. Such passaging techniques can be performed with any known tumor or cancer cell lines.

For example, Meth A, CMS4, CMS5, CMS21, and WEHI-164 are chemically induced fibrosarcomas of BALB/c female mice (DeLeo *et al.*, J. Exp. Med. 146, 720 [1977]), which provide a highly controllable model system for studying the anti-tumor activities of various agents (Palladino *et al.*, J. Immunol. 138, 4023-4032 [1987]). Briefly, tumor cells are propagated *in vitro* in cell culture. Prior to injection into the animals, the cell lines are washed and suspended in buffer, at a cell density of about $10x10^6$ to $10x10^7$ cells/ml. The animals are then infected subcutaneously with 10 to 100 µl of the cell suspension, allowing one to three weeks for a tumor to appear.

In addition, the Lewis lung (3LL) carcinoma of mice, which is one of the most thoroughly studied experimental tumors, can be used as an investigational tumor model. Efficacy in this tumor model has been correlated with beneficial effects in the treatment of human patients diagnosed with small cell carcinoma of the lung (SCCL). This tumor can be introduced in normal mice upon injection of tumor fragments from an affected mouse or of cells maintained in culture (Zupi *et al.*, Br. J. Cancer 41, suppl. 4, 309 [1980]), and evidence indicates that tumors can be started from injection of even a single cell and that a very high proportion of infected tumor cells survive. For further information about this tumor model see Zacharski, Haemostasis 16, 300-320 [1986]).

One way of evaluating the efficacy of a test compound in an animal model is implanted tumor is to measure the size of the tumor before and after treatment. Traditionally, the size of implanted tumors has been measured with a slide caliper in two or three dimensions. The measure limited to two dimensions does not accurately reflect the size of the tumor, therefore, it is usually converted into the corresponding volume by using a mathematical formula. However, the measurement of tumor size is very inaccurate. The therapeutic effects of a drug candidate can be better described as treatment-induced growth delay and specific growth delay. Another important variable in the description of tumor growth is the tumor volume doubling time. Computer programs for the calculation and description of tumor growth are also available, such as the program reported by Rygaard and Spang-Thomsen, *Proc. 6th Int. Workshop on Immune-Deficient Animals,* Wu and Sheng eds., Basel, 1989, 301. It is noted, however, that necrosis and inflammatory responses following treatment may actually result in an increase in tumor size, at least initially. Therefore, these changes need to be carefully monitored, by a combination of a morphometric method and flow cytometric analysis.

Recombinant (transgenic) animal models can be engineered by introducing the coding portion of the genes identified herein into the genome of animals of interest, using standard techniques for producing transgenic animals. Animals that can serve as a target for transgenic manipulation include, without limitation, mice, rats, rabbits, guinea pigs, sheep, goats, pigs, and non-human primates, e.g. baboons, chimpanzees and monkeys. Techniques known in the art to introduce a transgene into such animals include pronucleic microinjection (Hoppe and Wanger, U.S. Patent No. 4,873,191); retrovirus-mediated gene transfer into germ

lines (e.g., Van der Putten *et al.*, Proc. Natl. Acad. Sci. USA 82, 6148-615 [1985]); gene targeting in embryonic stem cells (Thompson *et al.*, Cell 56, 313-321 [1989]); electroporation of embryos (Lo, Mol. Cel. Biol. 3, 1803-1814 [1983]); sperm-mediated gene transfer (Lavitrano *et al.*, Cell 57, 717-73 [1989]). For review, see, for example, U.S. Patent No. 4,736,866.

For the purpose of the present invention, transgenic animals include those that carry the transgene only in part of their cells ("mosaic animals"). The transgene can be integrated either as a single transgene, or in concatamers, e.g., head-to-head or head-to-tail tandems. Selective introduction of a transgene into a particular cell type is also possible by following, for example, the technique of Lasko *et al.*, Proc. Natl. Acad. Sci. USA 89, 6232-636 (1992).

The expression of the transgene in transgenic animals can be monitored by standard techniques. For example, Southern blot analysis or PCR amplification can be used to verify the integration of the transgene. The level of mRNA expression can then be analyzed using techniques such as *in situ* hybridization, Northern blot analysis, PCR, or immunocytochemistry. The animals are further examined for signs of tumor or cancer development.

Alternatively, "knock out" animals can be constructed which have a defective or altered gene encoding a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide identified herein, as a result of homologous recombination between the endogenous gene encoding the polypeptide and altered genomic DNA encoding the same polypeptide introduced into an embryonic cell of the animal. For example, cDNA encoding a particular PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide can be used to clone genomic DNA encoding that polypeptide in accordance with established techniques. A portion of the genomic DNA encoding a particular PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and Capecchi, Cell, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see e.g., Li et al., Cell, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, by their ability to defend against certain pathological conditions and by their development of pathological conditions due to absence of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide.

The efficacy of antibodies specifically binding the polypeptides identified herein and other drug candidates, can be tested also in the treatment of spontaneous animal tumors. A suitable target for such studies is the feline oral squamous cell carcinoma (SCC). Feline oral SCC is a highly invasive, malignant tumor that is the most common oral malignancy of cats, accounting for over 60% of the oral tumors reported in this

species. It rarely metastasizes to distant sites, although this low incidence of metastasis may merely be a reflection of the short survival times for cats with this tumor. These tumors are usually not amenable to surgery, primarily because of the anatomy of the feline oral cavity. At present, there is no effective treatment for this tumor. Prior to entry into the study, each cat undergoes complete clinical examination, biopsy, and is scanned by computed tomography (CT). Cats diagnosed with sublingual oral squamous cell tumors are excluded from the study. The tongue can become paralyzed as a result of such tumor, and even if the treatment kills the tumor, the animals may not be able to feed themselves. Each cat is treated repeatedly, over a longer period of time. Photographs of the tumors will be taken daily during the treatment period, and at each subsequent recheck. After treatment, each cat undergoes another CT scan. CT scans and thoracic radiograms are evaluated every 8 weeks thereafter. The data are evaluated for differences in survival, response and toxicity as compared to control groups. Positive response may require evidence of tumor regression, preferably with improvement of quality of life and/or increased life span.

In addition, other spontaneous animal tumors, such as fibrosarcoma, adenocarcinoma, lymphoma, chrondroma, leiomyosarcoma of dogs, cats, and baboons can also be tested. Of these mammary adenocarcinoma in dogs and cats is a preferred model as its appearance and behavior are very similar to those in humans. However, the use of this model is limited by the rare occurrence of this type of tumor in animals.

8. Screening Assays for Drug Candidates

Screening assays for drug candidates are designed to identify compounds that bind or complex with the polypeptides encoded by the genes identified herein, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds, including peptides, preferably soluble peptides, (poly)peptide-immunoglobulin fusions, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

All assays are common in that they call for contacting the drug candidate with a polypeptide encoded by a nucleic acid identified herein under conditions and for a time sufficient to allow these two components to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, e.g. on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the polypeptide and drying. Alternatively, an immobilized antibody, e.g. a monoclonal antibody, specific for the polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, e.g. the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, e.g. by washing, and complexes anchored on the solid surface are detected. When the

originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labeled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide encoded by a gene identified herein, its interaction with that polypeptide can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers [Fields and Song, Nature (London) 340, 245-246 (1989); Chien *et al.*, Proc. Natl. Acad. Sci. USA 88, 9578-9582 (1991)] as disclosed by Chevray and Nathans [Proc. Natl. Acad. Sci. USA 89, 5789-5793 (1991)]. Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, while the other one functioning as the transcription activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of a GAL1-*lacZ* reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for β-galactosidase. A complete kit (MATCHMAKER™) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

Compounds that interfere with the interaction of a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2-encoding gene identified herein and other intra- or extracellular components can be tested as follows: usually a reaction mixture is prepared containing the product of the amplified gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a test compound to inhibit binding, the reaction is run in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture, to serve as positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described hereinabove. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

9. Compositions and Methods for the Treatment of Tumors

The compositions useful in the treatment of tumors associated with the amplification of the genes identified herein include, without limitation, antibodies, small organic and inorganic molecules, peptides, phosphopeptides, antisense and ribozyme molecules, triple helix molecules, etc. that inhibit the expression and/or activity of the target gene product.

For example, antisense RNA and RNA molecule act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. When antisense DNA is used,

oligodeoxyribonucleotides derived from the translation initiation site, e.g. between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details see, e.g. Rossi, Current Biology 4, 469-471 (1994), and PCT publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, e.g. PCT publication No. WO 97/33551, *supra*.

These molecules can be identified by any or any combination of the screening assays discussed hereinabove and/or by any other screening techniques well known for those skilled in the art.

9.1 Antibodies

Some of the most promising drug candidates according to the present invention are antibodies and antibody fragments which may inhibit the production or the gene product of the amplified genes identified herein and/or reduce the activity of the gene products.

i. Polyclonal Antibodies

Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

ii. Monoclonal Antibodies

The anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide, including fragments, or a fusion protein of such protein or a fragment thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of

human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection (ATCC), Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, supra]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for

example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison et al., supra] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

iii. Human and Humanized Antibodies

The anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibodies may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human

antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks *et al.*, Bio/Technology 10, 779-783 (1992); Lonberg *et al.*, Nature 368 856-859 (1994); Morrison, Nature 368, 812-13 (1994); Fishwild *et al.*, Nature Biotechnology 14, 845-51 (1996); Neuberger, Nature Biotechnology 14, 826 (1996); Lonberg and Huszar, Intern. Rev. Immunol. 13 65-93 (1995).

iv. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305:537-539 [1983]). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

v. Heteroconjugate Antibodies

Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

vi. Effector function engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance the effectiveness of the antibody in treating cancer, for example. For example cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, J. Exp Med. 176:1191-1195 (1992) and Shopes, B. J. Immunol. 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* Cancer Research 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, Anti-Cancer Drug Design 3:219-230 (1989).

vii. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.* an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y and ^{186}Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.* , Science 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.* avidin) which is conjugated to a cytotoxic agent (*e.g.* a radionucleotide).

viii. Immunoliposomes

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, Proc. Natl. Acad. Sci. USA, 82:3688 (1985); Hwang *et al.*, Proc. Natl Acad. Sci. USA, 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al* ., J. Biol. Chem. 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al.*., J. National Cancer Inst. 81(19)1484 (1989).

10. Pharmaceutical Compositions

Antibodies specifically binding the product of an amplified gene identified herein, as well as other molecules identified by the screening assays disclosed hereinbefore, can be administered for the treatment of tumors, including cancers, in the form of pharmaceutical compositions.

If the protein encoded by the amplified gene is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment which specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable region sequences of an antibody, peptide molecules can be designed which retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology (see, e.g. Marasco *et al.*, Proc. Natl. Acad. Sci. USA 90, 7889-7893 [1993]).

Therapeutic formulations of the antibody are prepared for storage by mixing the antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol,

trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN^{TM}, PLURONICS^{TM} or polyethylene glycol (PEG).

Non-antibody compounds identified by the screening assays of the present invention can be formulated in an analogous manner, using standard techniques well known in the art.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT ^{TM} (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

11. Methods of Treatment

It is contemplated that the antibodies and other anti-tumor compounds of the present invention may be used to treat various conditions, including those characterized by overexpression and/or activation of the amplified genes identified herein. Exemplary conditions or disorders to be treated with such antibodies and other compounds, including, but not limited to, small organic and inorganic molecules, peptides, antisense molecules, etc. include benign or malignant tumors (*e.g.* renal, liver, kidney, bladder, breast, gastric, ovarian, colorectal, prostate, pancreatic, ling, vulval, thyroid, hepatic carcinomas; sarcomas; glioblastomas; and various head and neck tumors); leukemias and lymphoid malignancies; other disorders such as neuronal, glial, astrocytal,

hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

The anti-tumor agents of the present invention, e.g. antibodies, are administered to a mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous administration of the antibody is preferred.

Other therapeutic regimens may be combined with the administration of the anti-cancer agents, e.g. antibodies of the instant invention. For example, the patient to be treated with such anti-cancer agents may also receive radiation therapy. Alternatively, or in addition, a chemotherapeutic agent may be administered to the patient. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *Chemotherapy Service* Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992). The chemotherapeutic agent may precede, or follow administration of the anti-tumor agent, e.g. antibody, or may be given simultaneously therewith. The antibody may be combined with an anti-oestrogen compound such as tamoxifen or an anti-progesterone such as onapristone (see, EP 616812) in dosages known for such molecules.

It may be desirable to also administer antibodies against other tumor associated antigens, such as antibodies which bind to theErbB2, EGFR, ErbB3, ErbB4, or vascular endothelial factor (VEGF). Alternatively, or in addition, two or more antibodies binding the same or two or more different antigens disclosed herein may be co-administered to the patient. Sometimes, it may be beneficial ω also administer one or more cytokines to the patient. In a preferred embodiment, the antibodies herein are co-administered with a growth inhibitory agent. For example, the growth inhibitory agent may be administered first, followed by an antibody of the present invention. However, simultaneous administration or administration of the antibody of the present invention first is also contemplated. Suitable dosages for the growth inhibitory agent are those presently used and may be lowered due to the combined action (synergy) of the growth inhibitory agent and the antibody herein.

For the prevention or treatment of disease, the appropriate dosage of an anti-tumor agent, e.g. an antibody herein will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the agent, and the discretion of the attending physician. The agent is suitably administered to the patient at one time or over a series of treatments.

For example, depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (*e.g.* 0.1-20mg/kg) of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

12. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the diagnosis or treatment of the disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for diagnosing or treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is usually an anti-tumor agent capable of interfering with the activity of a gene product identified herein, e.g. an antibody. The label on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

13. Diagnosis and Prognosis of Tumors

While cell surface proteins, such as growth receptors overexpressed in certain tumors are excellent targets for drug candidates or tumor (e.g. cancer) treatment, the same proteins along with secreted proteins encoded by the genes amplified in tumor cells find additional use in the diagnosis and prognosis of tumors. For example, antibodies directed against the proteins products of genes amplified in tumor cells can be used as tumor diagnostics or prognostics.

For example, antibodies, including antibody fragments, can be used to qualitatively or quantitatively detect the expression of proteins encoded by the amplified genes ("marker gene products"). The antibody preferably is equipped with a detectable, e.g. fluorescent label, and binding can be monitored by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. These techniques are particularly suitable, if the amplified gene encodes a cell surface protein, e.g. a growth factor. Such binding assays are performed essentially as described in section 5 above.

In situ detection of antibody binding to the marker gene products can be performed, for example, by immunofluorescence or immunoelectron microscopy. For this purpose, a histological specimen is removed from the patient, and a labeled antibody is applied to it, preferably by overlaying the antibody on a biological sample. This procedure also allows for determining the distribution of the marker gene product in the tissue examined. It will be apparent for those skilled in the art that a wide variety of histological methods are readily available for *in situ* detection.

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The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following

examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, VA. Unless otherwise noted, the present invention uses standard procedures of recombinant DNA technology, such as those described hereinabove and in the following textbooks: Sambrook *et al., Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Press N.Y., 1989; Ausubel *et al., Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y., 1989; Innis *et al., PCR Protocols: A Guide to Methods and Applications*, Academic Press, inc., N.Y., 1990; Harlow *et al., Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, 1988; Gait, M.J., *Oligonucleotide Synthesis*, IRL Press, Oxford, 1984; R.I. Freshney, *Animal Cell Culture*, 1987; Coligan *et al., Current Protocols in Immunology*, 1991.

EXAMPLE 1

Isolation of cDNA clones Encoding Human PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, and EBAF-2

1. Isolation of cDNA clones encoding human PRO187 (an FGF-8 homologue)

A proprietary expressed sequence tag (EST) DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA) was searched and an EST (#843193) was identified which showed homology to fibroblast growth factor (FGF-8) also known as androgen-induced growth factor.

mRNA was isolated from human fetal lung tissue using reagents and protocols from Invitrogen, San Diego, CA (Fast Track 2). The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents. (E.g., Invitrogen, San Diego, CA, Life Technologies, Gaithersburg, MD). The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into the cloning vector pRK5D using reagents and protocols from Life Technologies, Gaithersburg, MD (Super Script Plasmid System). The double stranded cDNA was sized to greater than 1000 bp and the SalI/NotI linkered cDNA was cloned into XhoI/NotI cleaved vector. pRK5D is a cloning vector that has an sp6 transcription initiation site followed by an SfiI restriction enzyme site preceding the XhoI/NotI cDNA cloning sites.

Several libraries from various tissue sources were screened by PCR amplification with the following oligonucleotide probes:

IN843193.f (OLI315) (SEQ ID NO: 55)
CAGTACGTGAGGGACCAGGGCGCCATGA

IN843193.r (OLI 317) (SEQ ID NO: 56)
CCGGTGACCTGCACGTGCTTGCCA

A positive library was then used to isolate clones encoding the FGF-8 homologue gene using one of the above oligonucleotides and the following oligonucleotide probe:

IN843193.p (OLI 316) (SEQ ID NO: 57)
GCGGATCTGCCGCCTGCTCANCTGGTCGGTCATGGCGCCCT

A cDNA clone was sequenced in entirety. The entire nucleotide sequence of FGF-8 homologue is shown in Figure 1 (SEQ ID NO: 1). Clone DNA27864 contains a single open reading frame with an apparent translational initiation site at nucleotide position 26 (Fig. 1; SEQ ID NO: 1). The predicted polypeptide precursor is 205 amino acids long. Clone DNA27864 has been deposited with the ATCC (designation: DNA27864-1155) on October 16, 1997, and is assigned ATCC deposit no. 209375.

2. Isolation of cDNA clones encoding human PRO533 (an FGF homologue)

The EST sequence accession number AF007268, a murine fibroblast growth factor (FGF-15) was used to search various public EST databases (e.g., GenBank, Dayhoff, etc.). The search was performed using the computer program BLAST or BLAST2 [Altschul et al., Methods in Enzymology, 266:460-480 (1996); http://blast.wustl/edu/blast/README.html] as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. The search resulted in a hit with GenBank EST AA220994, which has been identified as stratagene NT2 neuronal precursor 937230.

Based on this sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence. Forward and reverse PCR primers (notated as *.f and *.r, respectively) may range from 20 to 30 nucleotides (typically about 24), and are designed to give a PCR product of 100-1000 bp in length. The probe sequences (notated as *.p) are typically 40-55 bp (typically about 50) in length. In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification, as per *Ausubel et al., supra*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest by the in vivo cloning procedure suing the probe oligonucleotide and one of the PCR primers.

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO533 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal retina. The cDNA libraries used to isolated the cDNA clones were constructed by standard methods using commercially available reagents (e.g., Invitrogen, San Diego, CA; Clontech, etc.) The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

A cDNA clone was sequenced in its entirety. The full length nucleotide sequence of PRO533 is shown in Figure 5 (SEQ ID NO: 5). Clone DNA49435 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 459-461 (Fig. 5; SEQ ID NO: 5). The predicted polypeptide precursor is 216 amino acids long. Clone DNA49435-1219 has been deposited with ATCC (November 21, 1997) and is assigned ATCC deposit no. 209480.

Based on a BLAST-2 and FastA sequence alignment analysis of the full-length sequence, PRO533 shows amino acid sequence identity to fibroblast growth factor (FGF) (53%).

The oligonucleotide sequences used in the above procedure were the following:

FGF15.f: ATCCGCCCAGATGGCTACAATGTGTA (SEQ ID NO: 58)

FGF15.p: GCCTCCCGGTCTCCCTGAGCAGTGCCAAACAGCGGCAGTGTA (SEQ ID NO: 59)

FGF15.r: CCAGTCCGGTGACAAGCCCAAA (SEQ ID NO: 60)

PRO533 (UNQ334) encoded by DNA49435 has been identified as a specific ligand for FGF receptor 3, exhibiting exclusive binding to this receptor. As mice deficient in FGF receptor 3 are known to have major defects in bone growth and are deaf, PRO533 is anticipated to play a role in cartilage and bone growth and may be useful to prevent and/or treat hearing loss.

3. Isolation of cDNA clones encoding human PRO214 (an EGF-like homologue)

The extracellular domain (ECD) sequences (including the secretion signal sequence, if any) from about 950 known secreted proteins from the Swiss-Prot public database were used to search EST databases. The EST databases included public EST databases (e.g., GenBank), and a proprietary EST database (LIFESEQ^{TM}, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 [Altschul et al., Methods in Enzymology, 266:460-480 (1996); http://blast.wustl/edu/blast/README.html] as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons resulting in a BLAST score of 70 (or in some cases, 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington; http://bozeman.mbt.washington. edu/phrap.docs/phrap.html).

A consensus DNA sequence encoding EGF-like homologue was assembled using phrap. This consensus DNA sequence is indicated as DNA28744 (SEQ ID NO: 61) in Fig. 41.

Based on this consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence. Forward and reverse PCR primers (notated as *.f and *.r, respectively) may range from 20 to 30 nucleotides (typically about 24), and are designed to give a PCR product of 100-1000 bp in length. The probe sequences (notated as *.p) are typically 40-55 bp (typically about 50) in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than 1-1.5 kbp. In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification, as per *Ausubel et al., supra*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest by the in vivo cloning procedure suing the probe oligonucleotide and one of the PCR primers. The library used to isolate DNA32286 was human fetal lung.

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the EGF-like homologue gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from identify tissue type. The cDNA libraries used to isolated the cDNA clones were constructed by standard methods using commercially available reagents (e.g., Invitrogen, San Diego, CA; Clontech, etc.) The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

A cDNA clone was sequenced in its entirety. The full length nucleotide sequence of EGF-like homologue is shown in Figure 9 (SEQ ID NO: 9). DNA32286 contains a single open reading frame with an apparent translational initiation site at nucleotide position 103 (Fig. 9; SEQ ID NO: 9). The predicted polypeptide precursor is 420 amino acids long (Fig. 10, SEQ ID NO: 10).

Based on a BLAST and FastA sequence alignment analysis of the full-length sequence, EGF-like homologue DNA32286 shows amino acid sequence identity to HT protein and/or Fibulin (49% and 38%, respectively).

Clone DNA32286-1191 has been deposited with ATCC (October 16, 1997) and is assigned ATCC no. 209385.

The oligonucleotide sequences used in the above procedure were the following:

28744.p (OLI555)
CCTGGCTATCAGCAGGTGGGCTCCAAGTGTCTCGATGTGGATGAGTGTGA (SEQ ID NO: 62)

28744.f (OLI556)
ATTCTGCGTGAACACTGAGGGC (SEQ ID NO: 63)

28744.r (OLI557)
ATCTGCTTGTAGCCCTCGGCAC (SEQ ID NO: 64)

4. Isolation of cDNA clones encoding human PRO240 (a serrate homologue)

The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ^{TM}, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altshul et al., Methods in Enzymology 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

A consensus DNA sequence encoding PRO240 was assembled relative to the other identified EST sequences, wherein the consensus sequence was designated herein as DNA30873 (see Fig. 13, SEQ ID NO: 13). Based on this consensus sequence, oligonucleotides were synthesized to identify by PCR a cDNA library that contained the sequence of interest and for use as probes to isolate a clone of the full-length coding sequence for PRO240.

A pair of PCR primers (forward and reverse) were synthesized:

forward PCR primer 5'-TCAGCTCCAGACTCTGATACTGCC-3' (SEQ ID NO: 65)

reverse PCR primer 5'-TGCCTTTCTAGGAGGCAGAGCTCC-3' (SEQ ID NO: 66)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA30873 sequence which had the following nucleotide sequence

hybridization probe

5'-GGACCCAGAAATGTGTCCTGAGAATGGATCTTGTGTACCTGATGGTCCAG-3' (SEQ ID NO: 67)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO240 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal liver tissue. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., Science, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO240 [herein designated as UNQ214 (DNA34387)] and the derived protein sequence for PRO240.

The entire nucleotide sequence of UNQ214 (DNA34387) is shown in Figure 11 (SEQ ID NO: 11). Clone UNQ214 (DNA34387) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 12-14, and ending at the stop codon at nucleotide positions 699-701 (Fig. 11; SEQ ID NO: 11). The predicted polypeptide precursor is 229 amino acids long (Fig. 12, SEQ ID NO: 12). Clone UNQ214 (DNA34387) has been deposited with ATCC on September 17, 1997 and is assigned ATCC deposit no. 209260.

Analysis of the amino acid sequence of the full-length PRO240 suggests that it possesses 30% and 35% amino acid identity with the serrate precursor protein from *Drospohilia melanogaster* (SEQ ID NO: 15) and the C-serrate-1 protein from Gallus gallus (SEQ ID NO: 17), respectively (see Figures 14 and 15, respectively).

5. Isolation of cDNA clones encoding human PRO211 (an EGF-like homologue)

The extracellular domain (ECD) sequences (including the secretion signal sequence, if any) from about 950 known secreted proteins from the Swiss-Prot public database were used to search EST databases. The EST databases included public databases (e.g., Dayhoff, GenBank), and proprietary databases (e.g. LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altschul, SF and Gish (1996), *Methods in Enzymology* 266: 460-80 (1996); http://blast.wustl/edu/blast/README.html) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons with a Blast score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, WA; (http://bozeman.mbt.washington.edu/phrap.docs/phrap.html).

A consensus DNA sequences encoding an EGF-like homologue was assembled using phrap. The consensus DNA sequence (DNA28730) was extended by repeated cycles of blast and phrap as far as possible using the three sources of EST sequences listed above.

Based on this consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence. The pair of forward and reverse PCR primers (notated as *.f and *.r, respectively) may range from 20 to 30 nucleotides (typically 24), and are designed to give a PCR product of 100-1000 bp in length.

The probe sequences (notated as *.p) are typically 40-55 bp (typically 50) in length. In some cases additional oligonucleotides are synthesized when the consensus sequence is greater than 1-1.5 kbp. In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al., supra*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest by the *in vivo* cloning procedure using the probe oligonucleotide and one of the PCR primers. This library used to isolate DNA32292 fetal lung.

RNA for the construction of the cDNA libraries was isolated using standard isolation protocols, e.g., Ausubel *et al., supra*, from tissue or cell line sources or it was purchased from commercial sources (e.g., Clontech). The cDNA libraries used to isolate the cDNA clones were constructed by standard methods (e.g., Ausubel *et al.*) using commercially available reagents (e.g., Invitrogen). The cDNA was primed with oligo dT containing a Noti site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation in a suitable cloning vector (pRK5B or pRK5D) in the unique XhoI and NotI sites.

A cDNA clone was sequenced in its entirety. The entire nucleotide sequence (DNA32292) of an EGF-like homologue (PRO211) is shown in Figure 16 (SEQ ID NO: 18). The predicted polypeptide is 353 amino acids in length, respectively, with a molecule weight of approximately 38.19kDa. cDNA clone 32292-1131 has been deposited at ATCC (September 16, 1997) and assigned ATCC no. 209258.

The oligonucleotide sequences used in the above procedure were the following:

28730.p (OLI 516) (SEQ ID NO: 68)
AGGGAGCACGGACAGTGTGCAGATGTGGACGAGTGCTCACTAGCA

28730.f (OLI 517) (SEQ ID NO: 69)
AGAGTGTATCTCTGGCTACGC

28730.r (OLI 518) (SEQ ID NO: 70)
TAAGTCCGGCACATTACAGGTC

6. Isolation of cDNA clones encoding human PRO230 (having homology to tubulointerstitial nephritis antigen

The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ^{TM}, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altshul et al., Methods in Enzymology 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

A consensus DNA sequence encoding PRO230 was assembled relative to the other identified EST sequences, wherein the consensus sequence was designated herein as DNA30857 (SEQ. ID NO: 21) (see Figure19). An EST proprietary to Genentech was employed in the consensus assembly.

Based on the DNA30857 consensus sequence, oligonucleotides were synthesized to identify by PCR a cDNA library that contained the sequence of interest and for use as probes to isolate a clone of the full-length coding sequence for PRO230.

A pair of PCR primers (forward and reverse) were synthesized:

forward PCR primer 5'-TTCGAGGCCTCTGAGAAGTGGCCC-3' (SEQ ID NO: 71)

reverse PCR primer 5'-GGCGGTATCTCTCTGGCCTCCC-3' (SEQ ID NO: 72)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA30857 sequence which had the following nucleotide sequence

hybridization probe

5'-TTCTCCACAGCAGCTGTGGCATCCGATCGTGTCTCAATCCATTCTCTGGG-3' (SEQ ID NO: 73)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO230 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal lung tissue. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., Science, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO230 (herein designated as UNQ204 (DNA33223) and the derived protein sequence for PRO230.

The entire nucleotide sequence of UNQ204 (DNA33223) is shown in Figure 20 (SEQ ID NO: 22). Clone UNQ204 (DNA33223) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 100-103, and ending at the stop codon at nucleotide positions 1501-1503 (Figure 20 and SEQ ID NO: 22). The predicted polypeptide precursor is 467 amino acids long (Figure 18, SEQ ID NO: 20). A cDNA clone designated 32223-1136 has beeen deposited with ATCC (September 16, 1997), and was given ATCC accession no. 209264.

7. Isolation of cDNA clones encoding human PRO261 (a CTGF homologue/WISP-2)

The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ^{TM}, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altshul et al., Methods in Enzymology 266:460-

480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington; http://bozeman.mbt.washington.edu/phrap.docs/phrap.html).

A consensus DNA sequence was assembled relative to other EST sequences using phrap. This consensus sequence is herein designated DNA30843-from dna (see Figure 24, SEQ ID No: 28). Based on the DNA30843-from dna consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO261. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel et al., Current Protocols in Molecular Biology, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest by the *in vivo* cloning procedure using the probe oligonucleotide and one of the primer pairs.

A pair of PCR primers, forward (SEQ ID NO: 74) and reverse (SEQ ID NO: 75), were synthesized. Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA30843-from dna sequence which has the sequence of SEQ ID NO: 76.

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO261 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal lung tissue. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., Science, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO261 [herein designated as UNQ228 (DNA33473-seq min)] (Figure 22; SEQ ID NO: 25) and the derived protein sequence for PRO261 (SEQ ID NO: 27).

The synthetic oligonucleotides used in this process were:

30843.f: AAAGGTGCGTACCCAGCTGTGCC (SEQ ID NO: 74)

30843.r: TCCAGTCGGCAGAAGCGGTTCTGG (SEQ ID NO: 75)

30843.p: CCTGGTGCTGGATGGCTGTGGCTGCTGCCGGGTATGTGCACGGCGGCTGGG (SEQ ID NO: 76)

The entire nucleotide sequence of UNQ228 (DNA33473-seq min) is shown in Figure 22 (SEQ ID NO:SEQ ID NO: 25). Clone UNQ228 (DNA33473-seq min) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 1-3 of SEQ ID NO: 26 and ending at the stop codon

after nucleotide 750 of SEQ ID NO: 26. The predicted polypeptide precursor is 250 amino acids long (Figure 23). Clone UNQ228 (DNA33473-seq min) has been deposited with ATCC and is assigned ATCC deposit no. 209391.

Analysis of the amino acid sequence of the full-length PRO261 polypeptide suggests that portions of it possess significant homology to CTGF, thereby indicating that PRO261 is a novel growth factor.

8. Isolation or cDNA clones encoding human PRO246 (an HCAR homologue)

The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ^{TM}, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altshul et al., Methods in Enzymology 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

A consensus DNA sequence was assembled relative to other EST sequences using phrap. This consensus sequence is herein designated DNA30955 (Figure 28, SEQ ID NO: 34). Based on the DNA30955 consensus sequence, oligonucleotides were synthesized to identify by PCR a cDNA library that contained the sequence of interest and for use as probes to isolate a clone of the full-length coding sequence for PRO246.

A pair of PCR primers (forward and reverse) were synthesized:

forward PCR primer 5'-AGGGTCTCCAGGAGAAAGACTC-' (SEQ ID NO: 77)
reverse PCR primer 5'-ATTGTGGGCCTTGCAGACATAGAC-3' (SEQ ID NO: 78)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA30955 sequence which had the following nucleotide sequence

hybridization probe
5'-GGCCACAGCATCAAAACCTTAGAACTCAATGTACTGGTTCCTCCAGCTCC-3' (SEQ ID NO: 79)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO246 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal liver tissue. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., Science, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO246 [herein designated as UNQ220 (DNA35639)] (SEQ ID NO: 31) and the derived protein sequence for PRO246 (SEQ ID NO: 33).

The entire nucleotide sequence of UNQ220 (DNA35639) is shown in Figure 26 (SEQ ID NO: 31). Clone UNQ220 (DNA35639) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 126-128, and ending at the stop codon at nucleotide positions 1296-1298 (Fig. 26). The predicted polypeptide precursor is 390 amino acids long (Figure 27). Clone UNQ220 (DNA35639) has been deposited with ATCC on October 17, 1997 and is assigned ATCC deposit no. 209396.

Analysis of the amino acid sequence of the full-length PRO240 suggests that it possesses significant amino acid identity with the human cell surface protein HCAR as shown in Figure 29, thereby indicating that PRO246 may be a novel cell surface virus receptor.

9. Isolation of cDNA clones encoding human EBAF-2

The extracellular domain (ECD) sequences (including the secretion signal sequence, if any) from about 950 known secreted proteins from the SWISS-PROT™ public database were used to search EST databases. The EST databases included public EST databases (*e.g.*, GenBank), and a proprietary EST database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST™ or BLAST2™ (Altschul *et al.*, Methods in Enzymology, 266:460-480 (1996); http://blast.wustl/edu/blast/README.html) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons resulting in a BLAST™ score of 70 (or in some cases, 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington; http://bozeman.mbt.washington.edu/phrap.docs/phrap.html).

A consensus DNA sequence encoding EBAF-2 (DNA 28722) was assembled using phrap, as shown in Figure 31 (SEQ ID NO: 41). Based on this consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence. The forward and reverse PCR primers synthesized for this purpose were:

5'-AGGACTGCCATAACTTGCCTG (OLI489) (SEQ ID NO: 80) and
5'-ATAGGAGTTGAAGCAGCGCTGC (OLI490) (SEQ ID NO: 81).

The probe synthesized for this purpose was:

5'-TGTGTGGACATAGACGAGTGCCGCTACCGCTACTGCCAGCACCGC (OLI488) (SEQ ID NO: 82)

mRNA for construction of the cDNA libraries was isolated from human fetal kidney tissue (Invitrogen, San Diego, CA; Clontech, etc.) The RNA was used to generate an oligo-dT primed cDNA library in the cloning vector pRK5B using reagents and protocols from Life Technologies, Gaithersburg, MD (Super Script Plasmid System™). PRK5B is a cloning vector that has an sp6 transcription initiation site and unique

*XhoI/Not*I cDNA cloning sites. It is a precursor of pRK5D that does not contain the *Sfi*I site (Holmes *et al.*, Science, 253:1278-1280 (1991)).

In this procedure, the double-stranded cDNA was primed with oligo dT containing a *Not*I site, linked with blunt to *Sal*I hemikinased adaptors, cleaved with *Not*I, sized to greater than 1000 bp appropriately by gel electrophoresis, and cloned in a defined orientation into the *XhoI/Not*I-cleaved pRK5B vector. In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al., supra*, with the PCR primer pair identified above. A positive library was then used to isolate clones containing the *ebaf-1* gene using the probe oligonucleotide identified above and one of the PCR primers.

A cDNA clone was sequenced in its entirety. The entire nucleotide sequence of DNA33461 (encoding EBAF-2) is shown in Figure 30 (SEQ ID NO: 37). Clone DNA33461 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 68-70 (Fig. 30; SEQ ID NO: 37). The predicted polypeptide precursor is 366 amino acids long. The predicted signal sequence is amino acids 1-18 of Figure 30 (SEQ ID NO: 40). There is one predicted N-linked glycosylation site at amino acid residue 160. Clone DNA33461 (designated DNA33461-1199) has been deposited with ATCC (October 15, 1997) and is assigned ATCC deposit no. 209367.

Figure 32 shows the BLAST^{TM} score, match, and percent homology alignment between protein EBAF-2 (SEQ ID NO: 40), encoded by DNA33461 (SEQ ID NO: 37), and human EBAF-1 (SEQ ID NO: 42). Based on this BLAST^{TM} and FastA^{TM} sequence alignment analysis (using the ALIGN^{TM} computer program) of the full-length sequence, EBAF-2 shows the most amino acid sequence identity to EBAF-1 (92%). The results also demonstrate a significant homology between human EBAF-2 and mouse LEFTY protein.

EXAMPLE 2

Gene Amplification

This example shows that the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, and EBAF-2-encoding genes are amplified in the genome of certain human lung, colon and/or breast cancers and/or cell lines. Amplification is associated with overexpression of the gene product, indicating that the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, and EBAF-2 proteins are useful targets for therapeutic intervention in certain cancers such as colon, lung, breast and other cancers. Therapeutic agent may take the form of antagonists of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2-encoding genes, for example, murine-human chimeric, humanized or human antibodies against a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 (PRO317) polypeptide.

The starting material for the screen was genomic DNA isolated from a variety cancers. The DNA is quantitated precisely, *e.g.* fluorometrically. As a negative control, DNA was isolated from the cells of ten normal healthy individuals which was pooled and used as assay controls for the gene copy in healthy individuals (not shown). The 5' nuclease assay (for example, TaqMan^{TM}) and real-time quantitative PCR (for example, ABI Prizm 7700 Sequence Detection System^{TM} (Perkin Elmer, Applied Biosystems Division, Foster City, CA)), were used to find genes potentially amplified in certain cancers. The results were used to determine whether the DNA encoding PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246 or PRO317 is over-represented in any of the primary lung or colon cancers or cancer cell lines or breast cancer cell

lines that were screened. The primary lung cancers were obtained from individuals with tumors of the type and stage as indicated in Table 1. An explanation of the abbreviations used for the designation of the primary tumors listed in Table 1 and the primary tumors and cell lines referred to throughout this example has been given hereinbefore.

The results of the Taqman™ are reported in delta (Δ) CT units. One unit corresponds 1 PCR cycle or approximately a 2-fold amplification relative to normal, two units corresponds to 4-fold, 3 units to 8-fold amplification and so on. Quantitation was obtained using primers and a Taqman™ fluorescent prove derived from the PRO187-, PRO533-, PRO214-, PRO240-, PRO211-, PRO230-, PRO261-, PRO246- or PRO317-encoding gene. Regions of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246 or PRO317 which are most likely to contain unique nucleic acid sequences and which are least likely to have spliced out introns are preferred for the primer and probe derivation, *e.g.* 3-untranslated region. The sequences for the primers and probes (forward, reverse and probe) used for the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246 and PRO317 gene amplification were as follows:

PRO187 (DNA27864):

27864.tm.f

5'-GGCCTTGCAGACAACCGT-3' (SEQ ID NO: 83)

27864.tm.r

5'-CAGACTGAGGGAGATCCGAGA-3' (SEQ ID NO: 84)

27864.tm.p (OLI6914)

5'-GCAGATTTTGAGGACAGCCACCTCCA-3' (SEQ ID NO: 85)

PRO533 (DNA49435)

DNA49435.tm.f

5'GGGACGTGCTTCTACAAGAACAG-3' (SEQ ID NO: 86)

DNA49435.tm.r

5'-CAGGCTTACAATGTTATGATCAGACA-3' (SEQ ID NO: 87)

DNA49435.tm.p

5'-TATTCAGAGTTTTCCATTGGCAGTGCCAGTT-3' (SEQ ID NO: 88)

PRO214 (DNA32286)

DNA32286.3utr-5

5'-GGGCCATCACAGCTCCCT-3' (SEQ ID NO: 89)

DNA32286.3utr-3b

5'-GGGATGTGGTGAACACAGAACA-3' (SEQ ID NO: 90)

DNA32286.3utr-probe

5'-TGCCAGCTGCA TGCTGCCAGTT-3' (SEQ ID NO: 91)

PRO240 (DNA34387)

DNA34387.3utr-5

5'-GTCAGGGAGCTCTGCTTCCTAG-3' (SEQ ID NO: 92)

DNA34387.3utr-3

5'-AAT GGCGGCCTCA ACCTT-3' (SEQ ID NO: 93)

DNA34387.3utr-probe.rc

5'-CGAATCCACTGGCGAAAGATGCCTT-3' (SEQ ID NO: 94)

PRO211 (DNA32292)

DNA32292.3utr-5

5'-CAGAAGGATGTCCCGTGGAA-3' (SEQ ID NO: 95)

DNA32292.3utr-3

5'-GCCGCTGTCCACTGCAG-3' (SEQ ID NO: 96)

DNA32292.3utr-probe.rc

5'-GACGGC ATCCTCAGGG CCACA-3' (SEQ ID NO: 97)

PRO230 (DNA33223)

DNA33223.3utr-5

5'-GAAGAGCACAGCTGCAGATCC-3' (SEQ ID NO: 101)

DNA33223.3utr-3

5'-GAGGTGTCCTGGCTTTGGTAGT-3' (SEQ ID NO: 102)

DNA33223.3utr-probe

5'-CCTCTGGCGCCCCCACTCAA-3' (SEQ ID NO: 103)

An alternate set of primers and probes was also used for PRO230:

33223.tm.f3

5'GAGTGCGACATCGAGAGCTT-3' (SEQ ID NO: 98)

33323.tm.r3

5'-CCGCAGCCTCAGTGATGA-3' (SEQ ID NO: 99)

33323.tm.p3

5'-ATGTCCTCCATGCCCACGCG-3' (SEQ ID NO: 100)

PRO261 (DNA33473)

DNA33473.3utr-5

5'-TCTAGCCCACTCCCTGCCT-3' (SEQ ID NO: 104)

DNA33473.3utr-3

5'-GAAGTCGGAGAGAAAGCTCGC-3' (SEQ ID NO: 105)

DNA33473.3utr-probe

5'-CACACACAGCCTATATCAAACATGCACACG-3' (SEQ ID NO: 106)

PRO246 (DNA35639)

DNA35639.3utr-5

5'-GGCAGAGACTTCCAGTCACTGA-3' (SEQ ID NO: 107)

DNA35639.3utr-3

5'-GCCAAGGGTGGTGTTAGATAGG-3' (SEQ ID NO: 108)

DNA35639.3utr-probe

5'-CAGGCCCCCTTGATCTGTACCCCA-3' (SEQ ID NO: 109)

PRO317 (DNA33461)

DNA33461.tm.f

5'-CCAGGAGAGCTGGCGATG-3' (SEQ ID NO: 110)

DNA33461.tm.r

5'-GCAAATTCAG GGCTCACTAG AGA-3' (SEQ ID NO: 111)

DNA33461.tm.p

5'-CACAGAGCATTTGTCCATCAGCAGTTCAG-3' (SEQ ID NO: 112)

The 5' nuclease assay reaction is a fluorescent PCR-based technique which makes use of the 5' exonuclease activity of Taq DNA polymerase enzyme to monitor amplification in real time. Two oligonucleotide primers are used to generate an amplicon typical of a PCR reaction. A third oligonucleotide, or probe, is designed to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the TAQ DNA polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liberated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

The 5' nuclease procedure is run on a real-time quantitative PCR device such as the ABI Prism 7700TM Sequence Detection. The system consists of a thermocycler, laser, charge-coupled device (CCD) camera and computer. The system amplifies samples in a 96-well format on a thermocycler. During amplification, laser-induced fluorescent signal is collected in real-time through fiber optics cables for all 96 wells, and detected at the CCD. The system includes software for running the instrument and for analyzing the data.

5' Nuclease assay data are initially expressed as Ct, or the threshold cycle. This is defined as the cycle at which the reporter signal accumulates above the background level of fluorescence. The ΔCt values are used as quantitative measurement of the relative number of starting copies of a particular target sequence in a nucleic acid sample when comparing cancer DNA results to normal human DNA results.

Table 1 describes the stage, T stage and N stage of various primary tumors which were used to screen the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246 and PRO317 compounds of the invention.

Table 1

Primary Lung and Colon Tumor Profiles

Primary Tumor	Stage	Other Stage	Dukes Stage	T Stage	N Stage
Human lung tumor SqCCA (SRCC724) [LT1]	IB	--	--	T1	N1
Human lung tumor NSCCa (SRCC725) [LT1a]	IA	--	--	T3	N0
Human lung tumor AdenoCa (SRCC726) [LT2]	IB	--	--	T2	N0
Human lung tumor AdenoCa (SRCC727) [LT3]	IB	--	--	T1	N2
Human lung tumor SqCCq (SRCC728) [LT4]	IIB	--	--	T2	N0
Human lung tumor AdenoCa (SRCC729) [LT6]	IV	--	--	T1	N0
Human lung tumor Aden/SqCCa (SRCC730) [LT7]	IB	--	--	T1	N0
Human lung tumor AdenoCa (SRCC731) [LT9]	IIB	--	--	T2	N0
Human lung tumor SqCCa (SRCC732) [LT10]	IA	--	--	T2	N1
Human lung tumor AdenoCa (SRCC733) [LT11]	IB	--	--	T1	N1
Human lung tumor AdenoCa (SRCC734) [LT12]	IIA	--	--	T2	N0
Human lung tumor BAC (SRCC735) [LT13]	IB	--	--	T2	N0
Human lung tumor SqCCa (SRCC736) [LT15]	IB	--	--	T2	N0
Human lung tumor SqCCa (SRCC737) [LT16]	IB	--	--	T2	N0
Human lung tumor SqCCa (SRCC738) [LT17]	IIB	--	--	T2	N1
Human lung tumor SqCCa (SRCC739) [LT18]	IB	--	--	T2	N0
Human lung tumor SqCCa (SRCC740) [LT19]	IB	--	--	T2	N0
Human lung tumor LCCa (SRCC741) [LT21]	IIB	--	--	T3	N1
Human colon AdenoCa (SRCC742) [CT2]	--	M1	D	pT4	N0
Human colon AdenoCa (SRCC743) [CT3]		--	B	pT3	N0
Human colon AdenoCa (SRCC 744) [CT8]			B	T3	N0

Human colon AdenoCa (SRCC745) [CT10]			A	pT2	N0
Human colon AdenoCa (SRCC746) [CT12]		MO, R1	B	T3	N0
Human colon AdenoCa (SRCC747) [CT14]		pMO, RO	B	pT3	pN0
Human colon AdenoCa (SRCC748) [CT15]		M1, R2	D	T4	N2
Human colon AdenoCa (SRCC749) [CT16]		pMO	B	pT3	pN0
Human colon AdenoCa (SRCC750) [CT17]			C1	pT3	pN1
Human colon AdenoCa (SRCC751) [CT1]		MO, R1	B	pT3	N0
Human colon AdenoCa (SRCC752) [CT4]			B	pT3	M0
Human colon AdenoCa (SRCC753) [CT5]		G2	C1	pT3	pN0
Human colon AdenoCa (SRCC754) [CT6]		pMO, RO	B	pT3	pN0
Human colon AdenoCa (SRCC755) [CT7]		G1	A	pT2	pN0
Human colon AdenoCa (SRCC756) [CT9]		G3	D	pT4	pN2
Human colon AdenoCa (SRCC757) [CT11]			B	T3	N0
Human colon AdenoCa (SRCC758) [CT18]		MO, RO	B	pT3	pN0

DNA Preparation:

DNA was prepared from cultured cell lines, primary tumors, normal human blood. The isolation was performed using purification kit, buffer set and protease and all from Quiagen, according to the manufacturer's instructions and the description below.

Cell culture lysis:

Cells were washed and trypsinized at a concentration of 7.5×10^8 per tip and pelleted by centrifuging at 1000 rpm for 5 minutes at 4°C, followed by washing again with 1/2 volume of PBS recentrifugation. The pellets were washed a third time, the suspended cells collected and washed 2x with PBS. The cells were then suspended into 10 mL PBS. Buffer C1 was equilibrated at 4°C. Quiagen protease #19155 was diluted into 6.25 ml cold ddH_20 to a final concentration of 20 mg/ml and equilibrated at 4°C. 10 mL of G2 Buffer was prepared by diluting Quiagen RNAse A stock (100 mg/ml) to a final concentration of 200 µg/ml.

Buffer C1 (10 mL, 4°C) and ddH2O (40 mL, 4°C) were then added to the 10 mL of cell suspension, mixed by inverting and incubated on ice for 10 minutes. The cell nuclei were pelleted by centrifuging in a Beckman swinging bucket rotor at 2500 rpm at 4°C for 15 minutes. The supernatant was discarded and the nuclei were suspended with a vortex into 2 mL Buffer C1 (at 4°C) and 6 mL ddH_2O, followed by a second 4°C centrifugation at 2500 rpm for 15 minutes. The nuclei were then resuspended into the residual buffer using 200 µl per tip. G2 buffer (10 ml) was added to the suspended nuclei while gentle vortexing was applied. Upon completion of buffer addition, vigorous vortexing was applied for 30 seconds. Quiagen protease (200 µl, prepared as indicated above) was added and incubated at 50°C for 60 minutes. The incubation and centrifugation was repeated until the lysates were clear (*e.g.*, incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

Solid human tumor sample preparation and lysis:

Tumor samples were weighed and placed into 50 ml conical tubes and held on ice. Processing was limited to no more than 250 mg tissue per preparation (1 tip/preparation). The protease solution was freshly prepared by diluting into 6.25 ml cold ddH_2O to a final concentration of 20 mg/ml and stored at 4°C. G2 buffer (20 ml) was prepared by diluting DNAse A to a final concentration of 200 mg/ml (from 100 mg/ml stock). The tumor tissue was homogenated in 19 ml G2 buffer for 60 seconds using the large tip of the polytron in a laminar-flow TC hood to order to avoid inhalation of aerosols, and held at room temperature. Between samples, the polytron was cleaned by spinning at 2 x 30 seconds each in 2L ddH_20, followed by G2 buffer (50 ml). If tissue was still present on the generator tip, the apparatus was disassembled and cleaned.

Quiagen protease (prepared as indicated above, 1.0 ml) was added, followed by vortexing and incubation at 50°C for 3 hours. The incubation and centrifugation was repeated until the lysates were clear (*e.g.*, incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

Human blood preparation and lysis:

Blood was drawn from healthy volunteers using standard infectious agent protocols and citrated into 10 ml samples per tip. Quiagen protease was freshly prepared by dilution into 6.25 ml cold ddH_2O to a final concentration of 20 mg/ml and stored at 4°C. G2 buffer was prepared by diluting RNAse A to a final concentration of 200 µg/ml from 100 mg/ml stock. The blood (10 ml) was placed into a 50 ml conical tube and 10 ml C1 buffer and 30 ml ddH_2O (both previously equilibrated to 4°C) were added, and the components mixed by inverting and held on ice for 10 minutes. The nuclei were pelleted with a Beckman swinging bucket rotor at 2500 rpm, 4°C for 15 minutes and the supernatant discarded. With a vortex, the nuclei were suspended into 2 ml C1 buffer (4°C) and 6 ml ddH_2O (4°C). Vortexing was repeated until the pellet was white. The nuclei were then suspended into the residual buffer using a 200 µl tip. G2 buffer (10 ml) were added to the suspended nuclei while gently vortexing, followed by vigorous vortexing for 30 seconds. Quiagen protease was added (200 µl) and incubated at 50°C for 60 minutes. The incubation and centrifugation was repeated until the lysates were clear (*e.g.*, incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

Purification of cleared lysates:

(1) <u>Isolation of genomic DNA</u>:

Genomic DNA was equilibrated (1 sample per maxi tip preparation) with 10 ml QBT buffer. QF elution buffer was equilibrated at 50°C. The samples were vortexed for 30 seconds, then loaded onto equilibrated tips and drained by gravity. The tips were washed with 2 x 15 ml QC buffer. The DNA was eluted into 30 ml silanized, autoclaved 30 ml Corex tubes with 15 ml QF buffer (50°C). Isopropanol (10.5 ml) was added to each sample, the tubes covered with parafin and mixed by repeated inversion until the DNA precipitated. Samples were pelleted by centrifugation in the SS-34 rotor at 15,000 rpm for 10 minutes at 4°C. The pellet location was marked, the supernatant discarded, and 10 ml 70% ethanol (4°C) was added. Samples were pelleted again by centrifugation on the SS-34 rotor at 10,000 rpm for 10 minutes at 4°C. The pellet location was marked and the supernatant discarded. The tubes were then placed on their side in a drying rack and dried 10 minutes at 37°C, taking care not to overdry the samples.

After drying, the pellets were dissolved into 1.0 ml TE (pH 8.5) and placed at 50°C for 1-2 hours. Samples were held overnight at 4°C as dissolution continued. The DNA solution was then transferred

to 1.5 ml tubes with a 26 gauge needle on a tuberculin syringe. The transfer was repeated 5x in order to shear the DNA. Samples were then placed at 50°C for 1-2 hours.

Quantitation of genomic DNA and preparation for gene amplification assay:

The DNA levels in each tube were quantified by standard A260, A280 spectrophotometry on a 1:20 dilution (5 µl DNA + 95 µl ddH_2O) using the 0.1 ml quartz cuvetts in the Beckman DU640 spectrophotometer. A260/A280 ratios were in the range of 1.8-1.9. Each DNA samples was then diluted further to approximately 200 ng/ml in TE (pH 8.5). If the original material was highly concentrated (about 700 ng/µl), the material was placed at 50°C for several hours until resuspended.

Fluorometric DNA quantitation was then performed on the diluted material (20-600 ng/ml) using the manufacturer's guidelines as modified below. This was accomplished by allowing a Hoeffer DyNA Quant 200 fluorometer to warm-up for about 15 minutes. The Hoechst dye working solution (#H33258, 10 µl, prepared within 12 hours of use) was diluted into 100 ml 1 x TNE buffer. A 2 ml cuvette was filled with the fluorometer solution, placed into the machine, and the machine was zeroed. pGEM 3Zf(+) (2 µl, lot #360851026) was added to 2 ml of fluorometer solution and calibrated at 200 units. An additional 2 µl of pGEM 3Zf(+) DNA was then tested and the reading confirmed at 400 +/- 10 units. Each sample was then read at least in triplicate. When 3 samples were found to be within 10% of each other, their average was taken and this value was used as the quantification value.

The fluorometricly determined concentration was then used to dilute each sample to 10 ng/µl in ddH_2O. This was done simultaneously on all template samples for a single TaqMan plate assay, and with enough material to run 500-1000 assays. The samples were tested in triplicate with Taqman™ primers and probe both B-actin and GAPDH on a single plate with normal human DNA and no-template controls. The diluted samples were used provided that the CT value of normal human DNA subtracted from test DNA was +/- 1 CT. The diluted, lot-qualified genomic DNA was stored in 1.0 ml aliquots at -80°C. Aliquots which were subsequently to be used in the gene amplification assay were stored at 4°C. Each 1 ml aliquot is enough for 8-9 plates or 64 tests.

Gene amplification assay:

The PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246 and EBAF-2 (PRO317) compounds of the invention were screened in the following primary tumors and the resulting ΔCt values are reported in Table 2.

Table 2

ΔCt values in lung and colon primary tumor models

Primary Tumor	PRO187	PRO533	PRO214	PRO240	PRO211	PRO230	PRO261	PRO246	PRO317
LT1	-1.53	-0.05	0.14, 0.13	0.02	0.40, -0.48	-0.24	0.41	0.31, 0.85	0.52
LT1a	-0.17	1.02	0.85	1.36	1.18	0.45	1.08	0.89	1.29
LT2	-1.75	-0.17	-0.09	0.52	0.56	-0.24	0.27	-0.13	0.69
LT3	0.32	0.78	1.57	1.22	1.97	0.86	0.98	1.06	1.86
LT4	-1.80	0.14	0.28	0.13	1.17	-0.44	0.32	0.64	1.18
LT6	-0.12	-0.02	0.66	1.12	0.75	0.83	0.45	0.60	1.93
LT7	-1.09	1.04	0.87	0.89	0.51	0.73	0.03	0.10	1.52
LT9	-0.42	0.80	0.89	0.88	1.42	0.54	0.18	0.27	1.04
LT10	0.31	0.79	0.96	1.24	1.96	0.31	1.16	1.07	2.57
LT11	0.54, 0.22	1.09	1.67, 1.80	1.98, 2.38, 2.78, 1.99, 1.90	2.05, 1.89	1.32, 1.14, 0.83, 1.54, 0.80	0.67, 1.59, 0.63, 0.19,	3.43, 1.41	2.20, 2.33
LT12	2.70, 2.90	0.76	1.38, 1.49	2.32, 2.54, 2.42, 3.02, 3.07	1.51, 1.27	2.86, 2.96, 2.92, 2.68, 2.28	0.80, 1.73, 1.08, 2.23	2.47, 1.54	2.54, 1.74
LT13	2.74, 2.98	0.91	1.85, 1.83	2.01, 2.37, 2.13, 2.46, 2.58	1.88, 2.26	3.42, 3.22, 2.84, 2.75, 2.53	1.02, 1.13, 1.01, 0.29	1.63, 1.68	1.90, 2.24
LT15	3.75, 3.92	0.50	1.77, 1.58	2.44, 2.77, 2.79, 3.25, 3.30	2.44, 2.16	4.32, 4.47, 3.64, 3.56, 3.32	0.97, 2.64, 0.56, 2.38	2.11, 1.56	2.06, 2.76
LT16	-0.23, -0.28	1.66	0.91, 0.82	0.95, 1.11, 1.05, 1.37, 1.20	1.25, 0.86	1.15, 0.97, 0.29, 0.72, 0.54	0.80, 0.75, 0.82, 2.05	0.92, 0.18	1.55, 1.08
LT17	0.71, 0.47	1.32	1.93, 1.87	1.24, 2.47, 2.03, 1.95, 1.79	1.85, 2.30	1.26, 1.39, 1.30, 1.33, 1.30	1.67, 2.01, 1.43, 0.93	2.68, 1.69	2.29, 2.03

LT18	-1.29, -1.58	0.34	-0.11, -0.27	-0.44, 0.40, 0.07, 0.33, 0.30	0.48, 0.53	-0.21, -0.50 -1.19, 0.07, -0.44	1.22, 0.46, 0.15, -0.17	0.36, -0.28	-0.66 -0.38
LT19	4.05, 3.99	1.67	2.09, 1.98	3.07, 3.35, 3.64, 3.07, 3.44	2.42, 2.55	4.92, 4.93, 3.78, 4.76, 4.05	0.78, 1.38, 1.39, 2.33	1.91, 1.68	2.51, 2.03
LT21	-0.43, -0.72	0.92	0.97, 0.94	-0.04, 1.22, 0.80, 0.50, 0.52	1.26, 0.98	0.03, 0.44, -1.48, 0.49, 1.09	0.04, 1.14, 0.48, 3.40	1.50, 0.60	0.08, 0.45
CT2	--	--	2.49	1.89	--	0.82	1.66	--	--
CT3	--	--	2.06	1.65	--	1.34	2.14	--	--
CT8	--	--	1.48	1.21	--	0.60	0.55	--	--
CT10	--	--	1.84	1.81	--	1.00	1.00	--	--
CT12	--	--	1.81	1.37	--	1.13	0.34	--	--
CT14	--	--	2.48	2.20	--	0.79	1.03	--	--
CT15	--	--	1.63	1.85	--	0.96	0.67	--	--
CT16	--	--	1.95	1.83	--	1.40	0.87	--	--
CT17	--	--	2.04	1.76	--	1.74	-0.19	--	--
CT1	--	--	1.22	1.50	1.27	1.25	-0.06	--	--
CT4	--	--	1.36	1.86	1.33	1.32	1.00	--	--
CT5	--	--	1.56	2.41	1.76	2.27	1.07	--	--
CT6	--	--	1.33	1.58	1.01	0.97	-0.08	--	--
CT7	--	--	0.64	1.09	0.70	1.39	0.15	--	--
CT9	--	--	0.38	-0.05	0.21	-0.18	0.68	--	--
CT11	--	--	2.05	3.08	2.01	1.75	0.59	--	--
CT18	--	--	0.63	0.26	0.76	0.75	0.73	--	--
A549	--	--	-0.30	--	-0.71	--	--	-1.67	--
Calu-1	--	--	0.10	--	-0.40	--	--	-0.79	--
Calu-6	--	--	-0.62	--	-0.78	--	--	-0.80	--
H157	--	--	0.10	--	-0.70	--	--	-0.08	--
H441	--	--	-0.45	--	-0.44	--	--	-0.13	--
H460	--	--	-0.51	--	-0.61	--	--	-0.48	--

SKMES1	--	--	-0.09	--	0.31	--	--	0.85	--
SW900	--	--	-0.05	--	1.86	--	--	-0.32	--
SW480	--	--	--	--	--	--	0.62, 1.90, 1.20, 1.57, 1.68, 1.36, 1.59, 1.86, 1.91, 2.36, 1.68, 1.53, 2.50	--	--
SW620	--	--	--	--	--	--	0.66, 1.65, 1.85, 1.63, 1.61, 1.24, 1.52, 1.98, 1.57, 1.83, 1.41, 1.42, 1.59	--	--
Colo320	--	--	--	--	--	--	-0.33, 0.66, 0.48, 0.91, 0.72, 0.33, 2.49, 0.99, 1.06, 1.24, 1.04, 0.46, 0.27	--	--
HT29	--	--	--	--	--	--	0.46, 1.95, 1.61, 2.58, 1.49, 1.38, 1.40, 2.00, 2.59, 2.59, 1.39, 1.32	--	--

HM7	--	--	--	--	--	--	-0.70, 0.74, -0.29, 0.66, 0.27, 0.08, 0.54, 0.67, 0.64, 0.34, 0.09, 0.29, 0.21	--	--
WiDr	--	--	--	--	--	--	0.19, 1.64, 1.00, 1.71, 1.44, 1.57, 0.93, 1.84, 1.58, 0.91, 0.87	--	--
HCT116	--	--	--	--	--	--	0.25, 1.29, 1.04, 2.01, 1.29, 1.07, 1.08, 2.05, 1.81, 1.56, 1.05, 1.09, 0.96	--	--
SKCO1	--	--	--	--	--	--	0.73, 1.99, 1.33, 1.00, 1.33, 1.26, 1.19, 2.10, 1.50, 2.13, 1.33, 1.29	--	--

SW403			--	--	--	--	0.26, 1.98, 1.42, 2.20, 2.40, 1.50, 1.43, 2.15, 1.52, 1.67, 2.19, 1.40, 1.29	--	--
LS174T	--	--	--	--	--	--	1.48	--	--
HBL100	--	--	--	--	--	--	1.40	--	--
MB435s	--	--	--	--	--	--	1.43	--	--
T47D	--	--	--	--	--	--	0.38	--	--
MB468	--	--	--	--	--	--	-0.08	--	--
MB175	--	--	--	--	--	--	0.23	--	--
MB361	--	--	--	--	--	--	0.37	--	--
BT20	--	--	--	--	--	--	1.66	--	--
MCF7	--	--	--	--	--	--	0.53	--	--
SKBR3	--	--	--	--	--	--	1.73	--	--

PRO230:

PRO230 (DNA33223) was also reexamined with both framework and epicenter mapping. Selected tumors from the above initial screen were reexamined with both framework and epicenter mapping. Figure 42 and Table 3 indicate the chromosomal mapping of both the framework and epicenter markers that were used in the present example. The framework markers are located approximately every 20 megabases and were used to control aneuploidy.

Table 3
Framework Markers

Map Position	Stanford Human Genome Center Marker Name
A1	SHGC-33169
A40	SHGC-3901
A84	AFM234tb6
A129	ACT1B03
A180	SHGC-34001
A220	AFM338wb5
A263	EST00691

A312	SHGC-7599
A355	SHGC-32839
A398	SHGC-37552
A443	SHGC-11921
A485	SHGC-9327
A520	SHGC-30345
A553	SHGC-3055

The ΔCt values of the above described framework markers along Chromosome 1 relative for PRO230 are indicated for selected tumors in Table 4.

Table 4
Amplification of framework markers relative to DNA33223 - Framework Markers (ΔCt)

	Framework Markers														
Tumor	A1	A40	A84	A129	A180	A220	A263	A312	A355	A398	A443	A484	A520	A553	DNA 33223-utr
LT1	0.64	0.11	0.03	-0.19	---	0.35	--	0.11	0.23	0.22	0.58	0.42	0.31	0.20	-0.38
LT1a	0.76	0.32	-0.18	-0.28	---	0.29	---	-0.54	-0.04	-4.27	-0.08	0.22	0.05	0.11	0.45
LT2	0.54	0.48	-0.03	-0.14	---	0.33	---	0.07	0.19	0.46	0.30	0.42	0.07	0.27	-0.32
LT3	1.14	0.51	-0.13	-0.21	---	0.65	---	-0.15	-0.05	-0.34	0.00	0.01	-0.05	0.02	0.87
LT4	0.73	0.21	-0.40	-0.10	---	0.45	---	-0.25	0.32	0.37	0.20	0.42	0.05	0.23	-0.63
LT6	0.55	0.44	-0.40	0.02	---	0.63	---	-0.56	0.43	0.83	-0.17	0.02	-0.16	-0.31	0.63
LT7	0.53	-0.04	-0.15	-0.25	---	0.63	---	-0.27	0.56	-0.58	0.87	0.96	0.70	0.54	0.86
LT9	0.55	-0.31	-0.08	-0.28	---	0.51	---	-0.63	-0.01	-0.12	-0.17	0.01	-0.30	-0.25	-0.07
LT10	0.78	0.11	0.16	0.11	---	0.90	---	-0.18	-0.18	0.00	0.15	-0.15	-0.09	-0.22	0.77
LT11	0.63	0.32	-0.48	-0.16	0.00	0.07	0.00	-0.15	0.18	0.13	-0.11	0.35	0.35	0.00	1.30
LT12	0.29	0.30	-0.32	-0.51	0.00	-0.18	0.00	-0.72	0.04	0.02	-0.21	-0.06	0.27	-0.10	2.76
LT13	0.66	0.39	-0.04	-0.47	0.00	-0.65	0.00	-0.04	-0.12	-0.24	-0.20	0.01	0.02	-0.08	3.42
LT15	0.53	0.29	0.00	-0.16	0.00	0.25	0.00	-0.38	-0.02	-0.03	-0.11	-0.11	0.65	0.18	4.64
LT16	0.89	0.57	0.73	-0.22	0.00	0.64	0.00	-0.97	0.37	0.12	-0.04	-0.03	0.83	0.35	0.73
LT17	0.66	0.41	-0.51	0.04	0.00	0.69	0.00	-0.22	0.09	0.22	-0.01	-0.25	0.60	0.26	1.24
LT18	-0.21	0.56	-0.11	-1.99	0.00	0.32	0.00	-0.83	0.02	-0.19	-0.04	-0.22	0.63	-0.01	-0.24
LT19	0.26	-13.38	-0.17	-0.31	0.00	0.02	0.00	-0.51	-0.10	0.28	-0.41	-0.29	0.41	0.04	4.74
LT21	0.37	0.23	-0.17	-0.09	0.00	0.69	0.00	-0.09	-0.10	-0.08	-0.04	-0.17	0.61	-0.07	0.28

Table 5 describes the epicenter markers that were employed in association with PRO230 (DNA33223). These markers are located in close proximity to DNA33223 and are used to assess the amplification status of the region of chromosome 1 in which DNA33223 is located. The distance between individual markers is measured in centirays, which is a radiation breakage unit approximately equal to a 1% chance of a breakage between two markers. One cR is very roughly equivalent to 20 kilobases. The marker SHGC-35321 is the marker found to be the closest to the location on chromosome 1 where DNA33223 most closely maps.

Table 5
Epicenter Markers

Map Position on Chromosome 1	Stanford Human Genome Center Marker Name	CR Distance to Next Marker
A83	SHGC-37693	97 (gap)
A84	AFM234tb6	9
A85	AFM240za9	46
A86	AFM199zd2	10
A87	SHGC-35321	11
A88	SHGC-3252	12
A89	SHGC-11204	173
A102	SHGC-11219	-

Table 6 indicates the ΔCt values for results of epicenter mapping relative to DNA33223, indicating the relative amplification in the region more immediate to the actual location of DNA33223 along chromosome 1.

Table 6
Amplification of Epicenter Markers Relative to DNA33223

	A83	A84	A85	A86	A87	A88	A89	A102	DNA33223.3'utr	DNA33223.tm3
LT1	-0.22	-0.05	-0.15	-0.05	1.14	-0.82	0.12	-0.07	-1.42	-0.58
LT1a	-0.35	-1.23	-0.48	0.02	1.32	0.90	-0.27	0.69	2.11	2.02
LT2	-0.23	-1.70	-0.32	-0.05	0.97	0.72	-0.03	0.32	-1.35	-0.73
LT3	0.09	-0.60	0.00	-0.26	1.53	1.17	0.22	1.01	1.51	1.68
LT4	-0.05	-0.44	-0.15	-0.08	0.90	0.90	-0.02	0.6	-1.70	-0.66
LT6	-0.20	-0.10	-0.19	-0.11	1.67	1.20	0.27	1.4	3.79	3.52
LT7	-0.28	0.22	-0.09	0.26	1.59	0.90	0.09	-14.51	0.97	0.73
LT9	-0.22	0.02	-0.23	0.07	1.90	1.43	0.04	2.02	4.44	4.26

	LT10	-0.29	-1.07	-0.27	-0.09	2.09	1.19	0.09	1.85	4.41	4.19
	LT11	-0.15	-0.02	-0.09	0.43	0.65	1.15	0.10	0.88	0.90	0.91
	LT12	-0.03	0.09	-0.20	0.16	0.59	1.00	0.13	0.36	2.39	1.74
	LT13	-0.01	-0.17	-0.25	0.23	0.68	1.06	0.06	0.81	2.70	2.15
5	LT15	-0.02	0.14	-0.18	-0.02	0.34	0.96	0.07	1.28	3.89	2.91
	LT16	0.22	0.49	0.43	0.47	0.94	1.12	0.34	1.37	0.18	0.31
	LT17	0.13	0.85	0.21	0.04	1.06	0.39	0.30	0.98	0.63	0.95
	LT18	-0.07	0.36	0.14	-0.11	0.47	-4.77	0.27	0.09	-0.82	-0.66
	LT19	-0.29	-0.44	-0.35	-0.30	0.19	-0.13	0.17	1.35	4.09	3.23
10	LT21	-0.22	-0.59	-0.13	-0.16	0.88	0.91	-0.05	0.44	-0.54	0.29

PRO240:

PRO240 was also reexamined with epicenter mapping. Table 7 describes the epicenter markers that were employed in association with PRO240 (DNA34387). These markers are located in close proximity to DNA34387 and are used to assess the amplification status of the region of chromosome 2 in which DNA34387 is located. The distance between individual markers is measured in centirays, which is a radiation breakage unit approximately equal to a 1% chance of a breakage between two markers. One cR is very roughly equivalent to 20 kilobases. The marker SHGC-14626 is the marker along chromosome 2 which most closely maps to DNA34387; however, the Taqman™ primers and probes for SHGC-1426 failed in our assay, due to technical difficulties related to PCR. DNA34387 was also found to be contained with a BAC (Bacterial Artificial Chromosome). The full BAC was about 100 Kb. The 5' and 3' ends were sequenced and used to generated the BAC primers indicated in Table 8. This confirms the validity of our original epicenter mapping results.

Table 7
Epicenter Markers Stanford Human Genome

Map Position on Chromosome 2	Stanford Human Genome Center Marker Name	Distance to next marker (cR)
B60	AFMa136wh9	65 (gap)
B63	AFM254vc9	6
B64	SHGC-14574	28
B65	SHGC-14626	7
B66	SHGC-11736	7
B67	SHGC-35430	17
B68	AFM234YA9	32
B71	CHLC.GATA8F07.440	--

Table 8 indicates the DCt values for results of epicenter mapping relative to DNA34387, indicating the relative amplification in the immediate chromosomal region along Chromosome 2.

Table 8

Amplification of Epicenter and BAC Markers Relative DNA34387

Framework Markers (ΔCt)

Lung tumor	B63	B64	B66	B67	B68	BAC (for.)	BAC (rev.)	DNA34387
LT1	0.22	0.34	0.10	0.65	0.64	0.11	0.91	0.65
LT1a	-0.04	0.03	-0.21	-0.03	0.22	1.29	0.61	1.63
LT2	0.15	-0.01	0.14	0.43	0.49	0.08	0.68	0.54
LT3	0.04	0.07	-0.30	0.16	0.29	0.98	0.89	1.97
LT4	-0.13	-0.09	-0.26	0.70	0.82	-0.26	0.82	0.70
LT6	-0.41	0.07	-0.71	0.06	0.35	0.81	0.69	1.26
LT7	-0.29	0.22	-0.32	0.29	0.32	0.74	0.42	1.06
LT9	-0.21	0.66	-0.15	0.38	0.32	0.62	0.48	1.58
LT10	-0.32	0.18	-0.28	0.76	0.28	0.99	0.38	1.41
LT11	0.08	0.76	0.24	0.05	0.88	2.13	0.72	2.25
LT12	-0.11	0.38	0.18	0.27	0.70	2.86	0.42	3.11
LT13	0.03	0.46	-0.02	0.00	0.68	2.84	0.53	2.85
LT15	-0.03	0.35	0.00	-0.08	0.52	3.73	0.71	3.78
LT16	-0.03	-16.55	0.02	0.23	0.63	0.91	0.47	1.33
LT17	0.20	0.45	0.19	0.58	0.86	1.99	0.80	2.20
LT18	-0.09	-0.45	0.11	0.46	0.32	-0.39	0.57	0.18
LT19	-0.18	0.32	-0.10	0.30	0.66	3.91	0.58	3.93
LT21	-0.02	0.40	-0.06	0.45	0.77	0.66	0.71	1.10

DISCUSSION and CONCLUSION:

PRO187:

The ΔCt values for DNA27864 (PRO187) in a variety of lung tumors are reported in Table 2. A ΔCt value of > 1 was typically used as the threshold value for amplification scoring, as this represents a doubling of the gene copy. Table 2 indicates that significant amplification of DNA 27864 occurred in primary lung tumors LT12, LT13, LT15, and LT19. The average ΔCt values were 2.80, 2.86, 3.84 and 4.02. This represents approximately a 7.0, 7.3, 14.3 and 16.2 fold increase in gene copy relative to normal tissue. Because amplification of DNA27864 (PRO187) occurs in various tumors, it is likely associated with tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA27864 (PRO187) would be expected to be useful in cancer therapy.

PRO533:

The ΔCt values for DNA49435 (PRO533) in a variety of lung tumors are reported in Table 2. A ΔCt value of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of the gene copy. Table 2 indicates that amplification of DNA49435 occurred in primary lung tumors LT1a, LT7, LT11, LT16, LT17 and LT19. The ΔCt values in these tumors were 1.02, 1.04, 1.09, 1.66, 1.32 and 1.67. This represents approximately a 2.0, 2.1, 2.1, 3.2, 2.5 and 3.2, respectively, fold increase in gene copy relative to normal tissue. Because amplification of DNA49435 (PRO533) occurs in various tumors, it is likely associated with tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA49435 (PRO533) would be expected to be useful in cancer therapy.

PRO214:

The ΔCt values for DNA32286 (PRO214) in a variety of lung and colon tumors are reported in Table 2. A ΔCt value of > 1 was typically used as the threshold value for amplification scoring, as this represents a doubling of the gene copy. Table 2 indicates that significant amplification of DNA32286 occurred in: (1) primary lung tumors LT3, LT11, LT12, LT13, LT15, LT17, LT19; and (2) primary colon tumors CT2, CT3, CT8, CT10, CT12, CT14, CT15, CT16, CT17, CT1, CT4, CT5, CT6 and CT11. The ΔCt and average ΔCt values for the lung tumors were the following: 1.57, 1.74, 1.44, 1.84, 1.68, 1.90, 2.04, respectively; while the those for the primary colon tumors were: 2.49, 2.06, 1.48, 1.84, 1.81, 2.48, 1.63, 1.95, 2.04, 1.22, 1.36, 1.56, 1.33 and 2.05, respectively. For the lung tumors, this represents approximately a 3.0, 3.3, 2.7, 3.6, 3.2, 3.7 and 4.1, respectively, fold increase in gene copy relative to normal tissue. For the colon tumors, this represents approximately a 5.6, 4.2, 2.8, 3.6, 3.5, 5.6, 3.1, 3.9, 4.1, 2.3, 2.6, 2.9, 2.5 and 4.1, respectively, fold increase in gene copy relative to normal tissue. Because amplification of DNA32286 (PRO214) occurs in various tumors, it is likely associated with tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA32286 (PRO214) would be expected to be useful in cancer therapy.

PRO240:

The ΔCt values for DNA34387 (PRO240) in a variety of lung and colon tumors are reported in Table 2. A ΔCt value of > 1 was typically used as the threshold value for amplification scoring, as this represents a doubling of the gene copy. Table 2 indicates that significant amplification of DNA34387 occurred in: (1) primary lung tumors LT1a, LT3, LT6, LT10, LT11, LT12, LT13, LT15, LT16, LT17 and LT19; and (2) primary colon tumors CT2, CT3, CT8, CT10, CT12, CT14, CT15, CT16, CT17, CT1, CT4, CT5, CT6, CT7 and CT11.

The ΔCt and average ΔCt values for the primary lung tumors were the following: 1.36, 1.22, 1.12, 1.24, 2.21, 2.67, 2.31, 2.91, 1.14, 1.90, 3.31, respectively; while those for the primary colon tumors were: 1.89, 1.65, 1.21, 1.81, 1.37, 2.20, 1.85, 1.83, 1.76, 1.50, 1.86, 2.41, 1.58, 1.09 and 3.08.

For the lung tumors, this represents approximately a 2.6, 2.3, 2.2, 2.4, 4.6, 6.4, 5.0, 7.5, 2.2, 3.7 and 9.9, respectively, fold increase in gene copy relative to normal tissue. For the colon tumors, this represents approximately a 3.7, 3.1, 2.3, 3.5, 2.6, 4.6, 3.6. 3.6, 3.4, 2.8, 3.6, 3.0, 2.1 and 8.5, respectively, fold increase in gene copy relative to normal tissue.

Additional epicenter mapping amplfication reported in Table 10 indicate that significant amplification of DNA34387 occured in primary lung tumors LT1a, LT3, LT6, LT7, LT9, LT10, LT11, LT12, LT13, LT15, LT16, LT17, LT19 and LT21, which report ΔCt's of 1.63, 1.97, 1.26, 1.06, 1.58, 1.41, 2.25, 3.11,

2.85, 3.78, 1.33, 2.20, 3.93 and 1.10, respectively. This represents approximately a 3.1, 3.9, 2.4, 2.1, 3.0, 2.6, 4.8, 8.6, 7.2, 13.7, 2.5, 4.6, 15.2 and 2.1, respectively, amplification in gene copy relative to normal tissue.

In contrast, the amplification of the closest known markers (Table 10) are not amplified to a greater extent than DNA34387. Moreover, it appers that DNA34387 is very close to the BAC marker 208K21For1, as the maker is amplification in the same pattern of tumors as DNA34387, and the degree of amplification is similar.

With the exception of 208K21For1, amplifiation of the closest markers to DNA34387 does not occur to a greater extent than that of DNA34387. This strongly suggests that DNA34387 is the gene that is the cause for the amplification of the particular region on Chromosome 2. Because amplification of DNA34387 (PRO240) occurs in various tumors, it is likely to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA34387 (PRO240) would be expected to be useful in cancer therapy.

Because amplification of DNA34387 (PRO240) occurs in various tumors, it is likely associated with tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA34387 (PRO240) would be expected to be useful in cancer therapy.

PRO211:

The ΔCt values for DNA32292 (PRO211) in a variety of primary lung and colon tumors as well as lung tumor cell lines are reported in Table 2. A ΔCt value of > 1 was typically used as the threshold value for amplification scoring, as this represents a doubling of the gene copy. Table 2 indicates that significant amplification of DNA32292 in: (1) primary lung tumors LT1a, LT3, LT4, LT9, LT10, LT11, LT12, LT13, LT15, LT16, LT17, LT19, LT21; (2) primary colon tumors CT1, CT4, CT5, CT6, CT11; and (3) lung tumor cell line SW900.

The ΔCt and average ΔCt values for the primary lung tumors were the following: 1.18, 1.97, 1.17, 1.42, 1.96, 1.97, 1.39, 2.07, 2.30, 1.06, 2.08, 2.49 and 1.12, respectively; while those for the primary colon tumors were: 1.27, 1.33, 1.76, 1.01 and 2.01; and the lung tumor cell line SW900 gave a ΔCt value of 1.86.

For the lung tumors, this represents approximately a 2.3, 3.9, 2.2, 2.7, 3.9, 3.9, 2.6, 4.2, 4.9, 2.1, 4.2, 5.6 and 2.2, respectively, fold increase in gene copy relative to normal tissue. For colon tumors, this represents approximately a 2.4, 2.5, 3.4, 2.0, 4.0, respectively, fold increase in gene copy relative to normal tissue. For the lung tumor cell line SW900, DNA32292 is present in approximately 3.6 fold above normal levels.

Because amplification of DNA32292 (PRO211) occurs in various tumors, it is likely associated with tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA32292 (PRO211) would be expected to be useful in cancer therapy.

PRO230:

The ΔCt values for DNA33223 (PRO230) in a variety of lung and colon tumors are shown in Table 2. PRO230 were subjected to further framework and epicenter analysis. A ΔCt value of > 1 was typically used as the threshold value for amplification scoring, as this represents a doubling of the gene copy. The framework markers analysis reports the relative amplification of particular regions of chromosome 1 in the indicated tumors, while the epicenter markers analysis gives a more precise reading of the relative amplification in the region immediately in the vicinity of the gene of interest.

Based on the initial screening data reported in Table 2 as well as framework and epicenter mapping data in Tables 3 and 5, DNA33223 (PRO230) shows amplification in lung tumors LT1a, LT3, LT6, LT9, LT10, LT12, LT13, LT15 and LT19. DNA33223 (PRO230) also shows amplification in colon tumors CT3, CT10, CT12, CT16, CT17, CT1, CT4, CT5, CT7 and CT11. The greatest amplification occurred in lung tumors LT6, LT9, LT10, LT12, LT13, LT15 and LT19, which reported confirmed average ΔCt values of 3.66, 4.35, 4.30, 2.07, 2.43, 3.40 and 3.66. This represents approximately a 12.6, 20.4, 19.7, 4.2, 5.4, 10.6 and 12.6, respectively, fold increase in gene copy in these samples relative to the normal samples. Confirmatory framework and epicenter amplification of the colon tumors was not performed, as amplification in the colon tumors did not appear to occur to as great an extent as in the lung.

In contrast, the amplification results for the closest known markers (Tables 4 and 6) are not amplified to a greater extend than DNA33223. This very strongly suggests that DNA33223 is the gene that is the cause for the amplification of that particular region on Chromosome 1. Because DNA33223 is the cause of amplification in a number of different tumors, it is likely to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA33223 (PRO230) would be expected to be useful in cancer therapy.

PRO261:

The ΔCt values for DNA33473 (PRO261) in a variety of primary lung and colon tumors as well as lung tumor cell lines are reported in Table 2. A ΔCt value of > 1 was typically used as the threshold value for amplification scoring, as this represents a doubling of the gene copy. Table 2 indicates that significant amplification of DNA33474 in: (1) primary lung tumors LT1a, LT10, LT12, LT15, LT17 and LT19; (2) primary colon tumors CT2, CT3, CT14, CT5; (3) colon tumor cell lines SW480, SW620, HT29, WiDr, HCT116, SKCO1, SW403, LS174T and (4) breast tumor cell lines HBL100, MB435s, BT20 and SKBR3.

The ΔCt and average ΔCt values for the primary lung tumors were the following: 1.08, 1.16, 1.17, 1.64, 1.50 and 1.47, respectively; those for the primary colon tumors were 1.16, 2.14, 1.03 and 1.07, respectively; those for the colon tumor cell lines 1.67, 1.54, 1.73, 1.24, 1.32, 1.35, 1.65, and 1.48, respectively; and those for the breast tumor cell lines were 1.40, 1.43, 1.66 and 1.73, respectively.

For the lung tumors, this represents approximately a 2.1, 2.2, 2.2, 3.1, 2.8 and 2.8, respectively, fold increase in gene copy relative to normal tissue. For the colon tumors, this represents 2.2, 4.4, 2.0 and 2.1, respectively, fold increase in gene copy relative to normal tissue. For the colon tumor cell lines, this represents a 3.2, 2.9, 3.3, 2.4, 2.5, 2.5, 3.1 and 2.8, respectively, fold increase in gene copy relative to normal tissue. For the breast tumor cell lines, this represents a 2.6, 2.7, 3.2 and 3.3, respectively, fold increase in gene copy relative to normal tissue. Because amplification of DNA33473 (PRO261) occurs in various tumors, it is likely associated with tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA33473 (PRO261) would be expected to be useful in cancer therapy.

PRO246:

The ΔCt values for DNA35639 (PRO246) in a variety of primary lung tumors and lung tumor cell lines are reported in Table 2. A ΔCt value of > 1 was typically used as the threshold value for amplification scoring, as this represents a doubling of the gene copy. Table 2 indicates that significant amplification of DNA35639 in primary lung tumors LT3, LT10, LT11, LT12, LT13, LT15, LT17, LT19 and LT21.

The ΔCt and average ΔCt values for the primary lung tumors were the following: 1.06, 1.07, 2.42, 2.00, 1.65, 1.83, 2.18, 1.79 and 1.05, which represents a 2.1, 2.1, 5.3, 4.0, 3.1, 3.6, 4.5, 3.5 and 2.1, respectively, fold increase in gene copy relative to normal tissue.

Because amplification of DNA35639 (PRO246) occurs in various tumors, it is likely associated with tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA35639 (PRO246) would be expected to be useful in cancer therapy.

EBAF-2 (PRO317):

The ΔCt values for DNA33461 (PRO317) in a variety of primary lung tumors and lung tumor cell lines are reported in Table 2. A ΔCt value of > 1 was typically used as the threshold value for amplification scoring, as this represents a doubling of the gene copy. Table 2 indicates significant amplification of DNA33461 in primary lung tumors LT1a, LT3, LT4, LT6, LT7, LT9, LT10, LT11, LT12, LT13, LT15, LT16, LT17 and LT19.

The ΔCt and average ΔCt values for the primary lung tumors were the following: 1.29, 1.86, 1.18, 1.93, 1.52, 1.04, 2.57, 2.26, 2.14, 2.07, 2.41, 1.31, 2.16 and 2.27, which represents a 2.4, 3.6, 2.3, 3.8, 2.9, 2.1, 5.9, 4.8, 4.4, 4.2, 5.3, 2.5, 4.5 and 4.8, respectively, fold increase in gene copy relative to normal tissue

Because amplification of DNA33461 (PRO317) occurs in various tumors, it is likely associated with tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA33461 (PRO317) would be expected to be useful in cancer therapy.

EXAMPLE 3

In situ Hybridization

In situ hybridization is a powerful and versatile technique for the detection and localization of nucleic acid sequences within cell or tissue preparations. It may be useful, for example, to identify sites of gene expression, analyze the tissue distribution of transcription, identify and localize viral infection, follow changes in specific mRNA synthesis and aid in chromosome mapping.

In situ hybridization was performed following an optimized version of the protocol by Lu and Gillett, Cell Vision 1: 169-176 (1994), using PCR-generated ^{33}P-labeled riboprobes. Briefly, formalin-fixed, paraffin-embedded human tissues were sectioned, deparaffinized, deproteinated in proteinase K (20 g/ml) for 15 minutes at 37°C, and further processed for *in situ* hybridization as described by Lu and Gillett, *supra*. A [^{33}-P] UTP-labeled antisense riboprobe was generated from a PCR product and hybridized at 55°C overnight. The slides were dipped in Kodak NTB2 nuclear track emulsion and exposed for 4 weeks.

^{33}P-Riboprobe synthesis

6.0 μl (125 mCi) of ^{33}P-UTP (Amersham BF 1002, SA<2000 Ci/mmol) were speed vac dried. To each tube containing dried ^{33}P-UTP, the following ingredients were added:

2.0 μl 5x transcription buffer

1.0 μl DTT (100 mM)

2.0 μl NTP mix (2.5 mM : 10 μl; each of 10 mM GTP, CTP & ATP + 10 μl H_2O)

1.0 μl UTP (50 μM)

1.0 μl Rnasin

1.0 μl DNA template (1μg)

1.0 μl H_2O

1.0 µl RNA polymerase (for PCR products T3 = AS, T7 = S, usually)

The tubes were incubated at 37°C for one hour. 1.0 µl RQ1 DNase were added, followed by incubation at 37°C for 15 minutes. 90 µl TE (10 mM Tris pH 7.6/1mM EDTA pH 8.0) were added, and the mixture was pipetted onto DE81 paper. The remaining solution was loaded in a Microcon-50 ultrafiltration unit, and spun using program 10 (6 minutes). The filtration unit was inverted over a second tube and spun using program 2 (3 minutes). After the final recovery spin, 100 µl TE were added. 1 µl of the final product was pipetted on DE81 paper and counted in 6 ml of Biofluor II.

The probe was run on a TBE/urea gel. 1-3 µl of the probe or 5 µl of RNA Mrk III were added to 3 µl of loading buffer. After heating on a 95°C heat block for three minutes, the gel was immediately placed on ice. The wells of gel were flushed, the sample loaded, and run at 180-250 volts for 45 minutes. The gel was wrapped in saran wrap and exposed to XAR film with an intensifying screen in -70°C freezer one hour to overnight.

^{33}P-Hybridization

Pretreatment of frozen sections The slides were removed from the freezer, placed on aluminium trays and thawed at room temperature for 5 minutes. The trays were placed in 55°C incubator for five minutes to reduce condensation. The slides were fixed for 10 minutes in 4% paraformaldehyde on ice in the fume hood, and washed in 0.5 x SSC for 5 minutes, at room temperature (25 ml 20 x SSC + 975 ml SQ H_2O). After deproteination in 0.5 µg/ml proteinase K for 10 minutes at 37°C (12.5 µl of 10 mg/ml stock in 250 ml prewarmed RNase-free RNAse buffer), the sections were washed in 0.5 x SSC for 10 minutes at room temperature. The sections were dehydrated in 70%, 95%, 100% ethanol, 2 minutes each.

Pretreatment of paraffin-embedded sections The slides were deparaffinized, placed in SQ H_2O, and rinsed twice in 2 x SSC at room temperature, for 5 minutes each time. The sections were deproteinated in 20 µg/ml proteinase K (500 µl of 10 mg/ml in 250 ml RNase-free RNase buffer; 37°C, 15 minutes) - human embryo, or 8 x proteinase K (100 µl in 250 ml Rnase buffer, 37°C, 30 minutes) - formalin tissues. Subsequent rinsing in 0.5 x SSC and dehydration were performed as described above.

Prehybridization The slides were laid out in plastic box lined with Box buffer (4 x SSC, 50% formamide) - saturated filter paper. The tissue was covered with 50 µl of hybridization buffer (3.75g Dextran Sulfate + 6 ml SQ H_2O), vortexed and heated in the microwave for 2 minutes with the cap loosened. After cooling on ice, 18.75 ml formamide, 3.75 ml 20 x SSC and 9 ml SQ H_2O were added, the tissue was vortexed well, and incubated at 42°C for 1-4 hours.

Hybridization 1.0 x 10^6 cpm probe and 1.0 µl tRNA (50 mg/ml stock) per slide were heated at 95°C for 3 minutes. The slides were cooled on ice, and 48 µl hybridization buffer were added per slide. After vortexing, 50 µl ^{33}P mix were added to 50 µl prehybridization on slide. The slides were incubated overnight at 55°C.

Washes Washing was done 2x10 minutes with 2xSSC, EDTA at room temperature (400 ml 20 x SSC + 16 ml 0.25M EDTA, V_f=4L), followed by RNaseA treatment at 37°C for 30 minutes (500 µl of 10 mg/ml in 250 ml Rnase buffer = 20 µg/ml), The slides were washed 2x10 minutes with 2 x SSC, EDTA at room temperature. The stringency wash conditions were as follows: 2 hours at 55°C, 0.1 x SSC, EDTA (20 ml 20 x SSC + 16 ml EDTA, V_f=4L).

DNA49435 (FGF homologue, FGF receptor 3 ligand)

Oligo A-251G 46mer: GGA TTC TAA TAC GAC TCA CTA TAG GGC GGA TCC TGG CCG GCC TCG G (SEQ ID NO: 43)

Oligo A-251H 48mer: CTA TGA AAT TAA CCC TCA CTA AAG GGA GCC CGG GCA TGG TCT CAG TTA (SEQ ID NO: 44)

Moderate expression was observed over cortical neurons in the fetal brain. Expression was observed over the inner aspect of the fetal retina, and possibly in the developing lens. Expression was seen over fetal skin, cartilage, small intestine, placental villi and umbilical cord. It adult tissues, there was an extremely high level of expression over the gallbladder epithelium (see Figure 34). Moderate expression was seen over the adult kidney, gastric and colonic epithelia. These data are consistent with the potential role of this molecule in cartilage and bone growth.

DNA32286 (EGF-like homologue)

Oligo B-138U 47mer: GGA TTC TAA TAC GAC TCA CTA TAG GGC CCC TCC TGC CTT CCC TGT CC (SEQ ID NO: 45)

Oligo A-134R 48mer: CTA TGA AAT TAA CCC TCA CTA AAG GGA GTG GTG GCC GCG ATT ATC TGC (SEQ ID NO: 46)

In fetal tissues, low level expression was observed throughout the mesenchyme. Moderate expression was seen in placental stromal cells in membraneous tissues (Figure 35), and in thyroid (Figure 36). Low level expression was seen in cortical neurons (Figure 37).

DNA 34387 (Jagged/EGF homologue)

Oligo B-231 W 48mer: GGA TTC TAA TAC GAC TCA CTA TAG GGC CCG AGA TAT GCA CCC AAT GTC (SEQ ID NO: 47)

Oligo B-231-X 47mer: CTA TGA AAT TAA CCC TCA CTA AAG GGA TCC CAG AAT CCC GAA GAA CA (SEQ ID NO: 48).

Elevated signal was observed at the following sites:

Fetal issues - thyroid epithelium, small intestinal epithelium, gonad, pancreatic epithelium, hepatocytes in liver and renal tubules. Expression was also seen in vascular tissue in developing bones.

Adult tissues - moderate signal in placental cytotrophoblast, renal tubular epithelium, bladder epithelium, parathyroid and epithelial tumors.

DNA33223 (tubulointerstitial nephritis antigen homologue)

Oligo DNA33223-p1: GGA TTC TAA TAC GAC TCA CTA TAG GGC GGC GAT GTC CAC TGG GGC TAC (SEQ ID NO: 49)

Oligo DNA33223-p2: CTA TGA AAT TAA CCC TCA CTA AAG GGA CGA GGA AGA TGG GCG GAT GGT (SEQ ID NO: 50)

Tissue sections showed an intense signal associated with arterial and venous vessels in the fetus. In arteries, the signal appeared to be confined to smooth-muscle/pericytic cells. (Figure 38.) The signal was also seen in capillary vessels and in glomeruli. Expression was also observed in epithelium cells in the fetal lens. Strong expression was also seen in cells within placental trophoblastic villi (Figure 34). These cells lie between the trophoblast and the fibroblast-like cells that express HGF, and have an uncertain histogenesis.

In the adult, there was no evidence of expression and the wall of the aorta and most vessels appeared to be negative. However, expression was seen over vascular channels in the normal prostate and in the epithelium lining the gallbladder. Some expression was seen in the vessels of the soft-tissue sarcoma and the renal cell carcinoma.

In summary, this molecule shows relatively specific vascular expression in the fetus as well as in some adult organs.

In a second experiment, vascular expression, similar to the foregoing results, was observed in fetal blocks. Expression was seen on vascular smooth muscle, rather than endothelium. Expression was also seen in smooth muscle of the developing oesophagus, hence this molecule is not vascular specific. Expression was examined in 4 lung and 4 breast carcinomas. Substantial expression was seen in vascular smooth muscle of at least 3 out of 4 lung cancers and 2 out of 4 breast cancers. In addition, in one breast carcinoma (IF97-06551 3E), expression was observed in peritumoral stromal cells of uncertain histogenesis (possibly myofibroblasts). No endothelial cell expression was observed in this study.

DNA33473 (CTGF homologue)

Oligo D-170R 45mer: GGA TTC TAA TAC GAC TCA CTA TAG GGC GCG AGG ACG GCG GCT TCA (SEQ ID NO: 51)

Oligo D-170V 48mer: CTA TGA AAT TAA CCC TCA CTA AAG GGA AGA GTC GCG GCC GCC CTT TTT (SEQ ID NO: 52)

Strong expression was observed in dermal fibroblasts in normal adult skin. Strong expression was seen, in two cirrhotic livers, at sites of active hepatic fibrosis. Moderate expression was found over fasiculata cells of adrenal cortex. This localization supports a role for this molecule in extracellular matrix formation or turnover.

DNA35639 (HCAR homologue)

Oligo B-258M 48mer: GGA TTC TAA TAC GAC TCA CTA TAG GGC TTG CTG CGG TTT TTG TTC CTG (SEQ ID NO: 53)

Oligo B-258N 48mer: CTA TGA AAT TAA CCC TCA CTA AAG GGA GCT GCC GAT CCC ACT GGT ATT (SEQ ID NO: 54)

This molecule was strongly expressed in fetal vascular endothelium, including tissues of the CNS (Figure 40). Lower level of expression was observed in adult vasculature, including CNS. It was no obviously expressed at higher levels in tumor vascular endothelium. Signal was also seen over bone matrix and adult spleen, however, this signal was not obviously cell associated.

EXAMPLE 4

Use of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 as a hybridization probe

The following method describes use of a nucleotide sequence encoding a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide as a hybridization probe.

DNA comprising the coding sequence of full-length or mature PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 (as shown in Figure 2, SEQ ID NO:2; Figure 6, SEQ ID NO: 6; Figure 10, SEQ ID NO: 10; Figure 12, SEQ ID NO: 12; Figure 17, SEQ ID NO: 19; Figure 18, SEQ ID NO: 20; Figure 23, SEQ ID NO: 27; Figure 27, SEQ ID NO: 33; and Figure 30, SEQ ID NO: 40) is

employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2) in human tissue cDNA libraries or human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2-derived probe to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 can then be identified using standard techniques known in the art.

EXAMPLE 5

Expression of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 in *E. coli*

This example illustrates preparation of an unglycosylated form of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 by recombinant expression in *E. coli*.

The DNA sequence encoding PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 (SEQ ID NOs: 1; 8; 12; 14; 16; 25; 29; 35; and 40, respectively) is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar et al., Gene, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 coding region, lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook et al., supra. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 protein can then be purified using a metal chelating column under conditions that allow tight binding of the protein.

PRO187 (UNQ161) and EBAF-2 (UNQ278) were expressed in *E. coli* in a poly-His tagged form, using the following procedure. The DNA encoding PRO187 or EBAF-2 was initially amplified using selected PCR primers. The primers contained restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector, and other useful sequences providing for efficient and reliable translation initiation, rapid purification on a metal chelation column, and proteolytic removal with enterokinase. The PCR-amplified, poly-His tagged sequences were then ligated into an expression vector, which was used to transform an *E. coli* host based on strain 52 (W3110 fuhA(tonA) lon galE rpoHts(htpRts) clpP(lacIq). Transformants were first grown in LB containing 50 mg/ml carbenicillin at 30°C with shaking until an O.D.600 of 3-5 was reached. Cultures were then diluted 50-100 fold into CRAP media (prepared by mixing 3.57 g $(NH_4)_2SO_4$, 0.71 g sodium citrate·2H2O, 1.07 g KCl, 5.36 g Difco yeast extract, 5.36 g Sheffield hycase SF in 500 mL water, as well as 110 mM MPOS, pH 7.3, 0.55% (w/v) glucose and 7 mM $MgSO_4$) and grown for approximately 20-30 hours at 30°C with shaking. Samples were removed to verify expression by SDS-PAGE analysis, and the bulk culture is centrifuged to pellet the cells. Cell pellets were frozen until purification and refolding.

E. coli paste from 0.5 to 1 L fermentations (6-10 g pellets) was resuspended in 10 volumes (w/v) in 7 M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate is added to make final concentrations of 0.1M and 0.02 M, respectively, and the solution was stirred overnight at 4°C. This step results in a denatured protein with all cysteine residues blocked by sulfitolization. The solution was centrifuged at 40,000 rpm in a Beckman Ultracentifuge for 30 min. The supernatant was diluted with 3-5 volumes of metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and filtered through 0.22 micron filters to clarify. Depending the clarified extract was loaded onto a 5 ml Qiagen Ni-NTA metal chelate column equilibrated in the metal chelate column buffer. The column was washed with additional buffer containing 50 mM imidazole (Calbiochem, Utrol grade), pH 7.4. The protein was eluted with buffer containing 250 mM imidazole. Fractions containing the desired protein were pooled and stored at 4°C. Protein concentration was estimated by its absorbance at 280 nm using the calculated extinction coefficient based on its amino acid sequence.

The proteins were refolded by diluting sample slowly into freshly prepared refolding buffer consisting of: 20 mM Tris, pH 8.6, 0.3 M NaCl, 2.5 M urea, 5 mM cysteine, 20 mM glycine and 1 mM EDTA. Refolding volumes were chosen so that the final protein concentration was between 50 to 100 micrograms/ml. The refolding solution was stirred gently at 4°C for 12-36 hours. The refolding reaction was quenched by the addition of TFA to a final concentration of 0.4% (pH of approximately 3). Before further purification of the protein, the solution was filtered through a 0.22 micron filter and acetonitrile was added to 2-10% final concentration. The refolded protein was chromatographed on a Poros R1/H reversed phase column using a mobile buffer of 0.1% TFA with elution with a gradient of acetonitrile from 10 to 80%. Aliquots of fractions with A280 absorbance were analyzed on SDS polyacrylamide gels and fractions containing homogeneous refolded protein were pooled. Generally, the properly refolded species of most proteins are eluted at the lowest concentrations of acetonitrile since those species are the most compact with their hydrophobic interiors shielded from interaction with the reversed phase resin. Aggregated species are usually eluted at higher acetonitrile concentrations. In addition to resolving misfolded forms of proteins from the desired form, the reversed phase step also removes endotoxin from the samples.

Fractions containing the desired folded PRO187 and EBAF-2 proteins, respectively, were pooled and the acetonitrile removed using a gentle stream of nitrogen directed at the solution. Proteins were formulated

into 20 mM Hepes, pH 6.8 with 0.14 M sodium chloride and 4% mannitol by dialysis or by gel filtration using G25 Superfine (Pharmacia) resins equilibrated in the formulation buffer and sterile filtered.

EXAMPLE 6

Expression of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 in mammalian cells

This example illustrates preparation of a potentially glycosylated form of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 by recombinant expression in mammalian cells.

The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 DNA using ligation methods such as described in Sambrook et al., supra. The resulting vector is called pRK5-PRO187, pRK5-PRO533, pRK5-PRO214, pRK5-PRO240, pRK5-PRO211, pRK5-PRO230, pRK5-PRO261, pRK5-PRO246, or pRK5-EBAF-2.

In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 µg pRK5-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 DNA is mixed with about 1 µg DNA encoding the VA RNA gene [Thimmappaya et al., Cell, 31:543 (1982)] and dissolved in 500 µl of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M $CaCl_2$. To this mixture is added, dropwise, 500 µl of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM $NaPO_4$, and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200 µCi/ml ^{35}S-cysteine and 200 µCi/ml ^{35}S-methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

In an alternative technique, PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 DNA may be introduced into 293 cells transiently using the dextran sulfate method described by Somparyrac et al., Proc. Natl. Acad. Sci., 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 µg pRK5- PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 µg/ml bovine insulin and 0.1 µg/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing

expressed PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 can be expressed in CHO cells. The pRK5- PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 vector can be transfected into CHO cells using known reagents such as $CaPO_4$ or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as ^{35}S-methionine. After determining the presence of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 can then be concentrated and purified by any selected method.

Epitope-tagged PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 may also be expressed in host CHO cells. The PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-His tag into a Baculovirus expression vector. The poly-His tagged PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 insert can then be subcloned into a SV40 driven vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 can then be concentrated and purified by any selected method, such as by Ni^{2+}-chelate affinity chromatography.

PRO214 (UNQ188); PRO240 (UNQ214); PRO211 (UNQ185); PRO230 (UNQ204); and PRO261 (UNQ228) were expressed in CHO cells by both a transient and a stable expression procedure. In addition, PRO246 (UNQ220) was transiently expressed in CHO cells.

Stable expression in CHO cells was performed using the following procedure. The proteins were expressed as an IgG construct (immunoadhesin), in which the coding sequences for the soluble forms (e.g. extracellular domains) of the respective proteins were fused to an IgG1 constant region sequence containing the hinge, CH2 and CH2 domains and/or is a poly-His tagged form.

Following PCR amplification, the respective DNAs were subcloned in a CHO expression vector using standard techniques as described in Ausubel et al., *Current Protocols of Molecular Biology*, Unit 3.16, John Wiley and Sons (1997). CHO expression vectors are constructed to have compatible restriction sites 5' and 3' of the DNA of interest to allow the convenient shuttling of cDNA's. The vector used expression in CHO cells is as described in Lucas *et al., Nucl. Acids Res.* 24: 9 (1774-1779 (1996), and uses the SV40 early promoter/enhancer to drive expression of the cDNA of interest and dihydrofolate reductase (DHFR). DHFR expression permits selection for stable maintenance of the plasmid following transfection.

Twelve micrograms of the desired plasmid DNA were introduced into approximately 10 million CHO cells using commercially available transfection reagents Superfect® (Quiagen), Dosper® or Fugene®

(Boehringer Mannheim). The cells were grown and described in Lucas *et al.*, supra. Approximately 3 x 10^{-7} cells are frozen in an ampule for further growth and production as described below.

The ampules containing the plasmid DNA were thawed by placement into water bath and mixed by vortexing. The contents were pipetted into a centrifuge tube containing 10 mLs of media and centrifuged at 1000 rpm for 5 minutes. The supernatant was aspirated and the cells were resuspended in 10 mL of selective media (0.2 μm filtered PS20 with 5% 0.2 μm diafiltered fetal bovine serum). The cells were then aliquoted into a 100 mL spinner containing 90 mL of selective media. After 1-2 days, the cells were transferred into a 250 mL spinner filled with 150 mL selective growth medium and incubated at 37°C. After another 2-3 days, a 250 mL, 500 mL and 2000 mL spinners were seeded with 3 x 10^5 cells/mL. The cell media was exchanged with fresh media by centrifugation and resuspension in production medium. Although any suitable CHO media may be employed, a production medium described in US Patent No. 5,122,469, issued June 16, 1992 was actually used. 3L production spinner is seeded at 1.2 x 10^6 cells/mL. On day 0, the cell number pH were determined. On day 1, the spinner was sampled and sparging with filtered air was commenced. On day 2, the spinner was sampled, the temperature shifted to 33°C, and 30 mL of 500 g/L glucose and 0.6 mL of 10% antifoam (e.g., 35% polydimethylsiloxane emulsion, Dow Corning 365 Medical Grade Emulsion). Throughout the production, pH was adjusted as necessary to keep at around 7.2. After 10 days, or until viability dropped below 70%, the cell culture was harvested by centrifugtion and filtering through a 0.22 μm filter. The filtrate was either stored at 4°C or immediately loaded onto columns for purification.

For the poly-His tagged constructs, the proteins were purified using a Ni-NTA column (Qiagen). Before purification, imidazole was added to the conditioned media to a concentration of 5 mM. The conditioned media was pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column was washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein was subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc containing) constructs of were purified from the conditioned media as follows. The conditioned medium was pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column was washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein was immediately neutralized by collecting 1 ml fractions into tubes containing 275 μL of 1 M Tris buffer, pH 9. The highly purified protein was subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity was assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation.

PRO187 (UNQ161); PRO533 (UNQ334); PRO214 (UNQ188): PRO240 (UNQ214); PRO211 (UNQ185); PRO230 (UNQ204); PRO261 (UNQ228); PRO246 (UNQ220), and EBAF-2 (UNQ278) were also produced by transient expression in COS cells.

EXAMPLE 7

Expression of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, and EBAF-2 in Yeast

The following method describes recombinant expression of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 from the ADH2/GAPDH promoter. DNA encoding PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2. For secretion, DNA encoding PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 signal peptide or other mammalian signal peptide, or, for example, a yeast alpha-factor or invertase secretory signal/leader sequence, and linker sequences (if needed) for expression of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 may further be purified using selected column chromatography resins.

EXAMPLE 8

Expression of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, and EBAF-2 in Baculovirus-Infected Insect Cells

The following method describes recombinant expression in Baculovirus-infected insect cells.

The sequence coding for PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-His tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 or the desired portion of the coding sequence of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 [such as the sequence encoding the extracellular domain of a transmembrane protein or the sequence encoding the mature protein if the protein is extracellular] is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley et al., Baculovirus expression vectors: A Laboratory Manual, Oxford: Oxford University Press (1994).

Expressed poly-His tagged PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 can then be purified, for example, by Ni^{2+}-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert et al., Nature, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM $MgCl_2$; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8) and filtered through a 0.45 μm filter. A Ni^{2+}-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A_{280} with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A_{280} baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot with Ni^{2+}-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His_{10}-tagged PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2, respectively are pooled and dialyzed against loading buffer.

Alternatively, purification of the IgG tagged (or Fc tagged) PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

PRO187 (UNQ161); PRO533 (UNQ334); PRO214 (UNQ188); PRO240 (UNQ214); PRO211 (UNQ185); PRO230 (UNQ204); PRO246 (UNQ220); and EBAF-2 (UNQ278) were expressed in baculovirus infected Sf9 insect cells. While the expression was actually performed in a 0.5-2 L scale, it can be readily scaled up for larger (e.g. 8 L) preparations. The proteins were expressed as an IgG construct (immunoadhesin), in which the protein extracellular region was fused to an IgG1 constant region sequence containing the hinge, CH2 and CH3 domains and/or in poly-His tagged forms.

Following PCR amplification, the respective coding sequences were subcloned into a baculovirus expression vector (pb.PH.IgG for IgG fusions and pb.PH.His.c for poly-His tagged proteins), and the vector and Baculogold® baculovirus DNA (Pharmingen) were co-transfected into 105 *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711), using Lipofectin (Gibco BRL). pb.PH.IgG and pb.PH.His are modifications of the commercially available baculovirus expression vector pVL1393 (Pharmingen), with modified polylinker regions to include the His or Fc tag sequences. The cells were grown in Hink's TNM-FH medium supplemented with 10% FBS (Hyclone). Cells were incubated for 5 days at 28°C. The supernatant was harvested and subsequently used for the first viral amplification by infecting Sf9 cells in Hink's TNM-FH medium supplemented with 10% FBS at an approximate multiplicity of infection (MOI) of 10. Cells were incubated for 3 days at 28°C. The

supernatant was harvested and the expression of the constructs in the baculovirus expression vector was determined by batch binding of 1 ml of supernatant to 25 mL of Ni-NTA beads (QIAGEN) for histidine tagged proteins or Protein-A Sepharose CL-4B beads (Pharmacia) for IgG tagged proteins followed by SDS-PAGE analysis comparing to a known concentration of protein standard by Coomassie blue staining.

The first viral amplification supernatant was used to infect a spinner culture (500 ml) of Sf9 cells grown in ESF-921 medium (Expression Systems LLC) at an approximate MOI of 0.1. Cells were incubated for 3 days at 28°C. The supernatant was harvested and filtered. Batch binding and SDS-PAGE analysis was repeated, as necessary, until expression of the spinner culture was confirmed.

The conditioned medium from the transfected cells (0.5 to 3 L) was harvested by centrifugation to remove the cells and filtered through 0.22 micron filters. For the poly-His tagged constructs, the protein construct were purified using a Ni-NTA column (Qiagen). Before purification, imidazole was added to the conditioned media to a concentration of 5 mM. The conditioned media were pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column was washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein was subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc containing) constructs of proteins were purified from the conditioned media as follows. The conditioned media were pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column was washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein was immediately neutralized by collecting 1 ml fractions into tubes containing 275 mL of 1 M Tris buffer, pH 9. The highly purified protein was subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity of the proteins was verified by SDS polyacrylamide gel (PEG) electrophoresis and N-terminal amino acid sequencing by Edman degradation.

EXAMPLE 9

Demonstration of binding of PRO533 (UNQ334) to FGF Receptor 3

PRO533 was expressed in baculovirus in a C-terminal His8 epitope tagged form as described in Example 8, as was a control C-terminal His8 epitope protein. The extracellular domains of FGF receptors 1-4 and TIE1 receptor were expressed as Fc fusion proteins. Proteins were allowed to interact in binding buffer (DMEM media + 10mM Hepes pH 7.4 + 0.1% albumin + 200 ng/ml heparin) at room temperature for one hour. Protein A Sepharose (Pharmacia) was added (0.01 ml) and binding continued for 30 minutes. Protein A Sepharose beads were collected and washed twice in binding buffer. Samples were then resolved by SDS PAGE under reducing conditions. Western blot analysis was conducted with anti-His antibody (Qiagen) as recommended by manufacturer. The results are shown in Figure 4. The specific binding components are as indicated above lanes 1-8 in Figure 4. Lane 9 contains PRO533-His (UNQ334-His) loaded directly onto gel for comparison. The position of the molecular weight markers is indicated on the left side of the gel for comparison.

The results demonstrate a high specificity binding to FGF Receptor 3 (FGFR3-Fc). This is very significant, since most FGF ligands bind more than one FGF receptor.

EXAMPLE 10

Preparation of Antibodies that Bind PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2

This example illustrates preparation of monoclonal antibodies which can specifically bind PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, supra. Immunogens that may be employed include purified PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2, fusion proteins containing PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2, and cells expressing recombinant PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibodies.

After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells will be screened in an ELISA for reactivity against PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, orEBAF-2. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 is within the skill in the art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

* * * *

Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC):

Material	ATCC Dep. No.	Deposit Date
DNA27864-1155	209375	October 16, 1997
DNA49435-1219	209480	November 21, 1997
DNA32286-1191	209385	October 16, 1997
DNA34387-1138	209260	September 16, 1997
DNA32292-1131	209258	September 16, 1997
DNA33223-1136	209264	September 16, 1997
DNA33473-1176	209391	October 17, 1997
DNA35639-1172	209396	October 17, 1997
DNA33461-1199	209367	October 15, 1997

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

Claims:

1. An isolated antibody binding a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide.

2. The antibody of claim 1 which induces death of a cell overexpressing a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide.

3. The antibody of claim 2 wherein said cell is a cancer cell.

4. The antibody of claim 1 which is a monoclonal antibody.

5. The antibody of claim 4, which has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues.

6. The antibody of claim 5 which is labeled.

7. The antibody of claim 6 which is immobilized on a solid support.

8. The antibody of claim 1 which is an antibody fragment, a single-chain antibody, or an anti-idiotypic antibody.

9. A composition comprising an antibody of claim 1 in admixture with a pharmaceutically acceptable carrier.

10. The composition of claim 9 comprising growth inhibitory amount of said antibody.

11. The composition of claim 10 further comprising a second antibody or a cytotoxic or chemotherapeutic agent.

12. A method for determining the presence of a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide comprising exposing a cell suspected of containing the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide to an anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody and determining binding of said antibody to said cell.

13. A method of diagnosing tumor in a mammal, comprising detecting the level of expression of a gene encoding a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher expression level in the test sample indicates the presence of tumor in the mammal from which the test tissue cells were obtained.

14. A method of diagnosing tumor in a mammal, comprising (a) contacting an anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody with a test sample of tissue cells obtained from the mammal , and (b) detecting the formation of a complex between the anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody and the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide in the test sample.

15. The method of claim 14 wherein said test sample is obtained from an individual suspected to have neoplastic cell growth or proliferation.

16. A cancer diagnostic kit, comprising an anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody and a carrier in suitable packaging.

17. The kit of claim 16 further comprising instructions for using said antibody to detect the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide.

18. A method for inhibiting the growth of tumor cells comprising exposing a cell which overexpresses a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide to an effective amount of an agent inhibiting the expression and/or activity of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide.

19. The method of claim 18 wherein said agent is an anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody.

20. The method of claim 19 wherein said tumor cells are further exposed to radiation treatment or a cytotoxic or chemotherapeutic agent.

21. An article of manufacture, comprising:

a container;

a label on the container; and

a composition comprising an active agent contained within the container; wherein the composition is effective for inhibiting the growth of tumor cells, the label on the container indicates that the composition can be used for treating conditions characterized by overexpression of a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide, and the active agent in the composition is an agent inhibiting the expression and/or activity of the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide.

22. The article of manufacture of claim 21 wherein said active agent is an anti-PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 antibody.

23. A method for identifying a compound capable of inhibiting the expression or activity of a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide, comprising contacting a candidate compound with a PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide under conditions and for a time sufficient to allow these two components to interact.

24. The method of claim 23 wherein said candidate compound or said PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide is immobilized on a solid support.

25. The method of claim 24 wherein the non-immobilized component carries a detectable label.

DNA27864

```
ATGGGAGCCG CCCGCCTGCT GCCCAACCTC ACTCTGTGCT TACAGCTGCT 50
GATTCTCTGC TGTCAAACTC AGTACGTGAG GGACCAGGGC GCCATGACCG 100
ACCAGCTGAG CAGGCGGCAG ATCCGCGAGT ACCAACTCTA CAGCAGGACC 150
AGTGGCAAGC ACGTGCAGGT CACCGGGCGT CGCATCTCCG CCACCGCCGA 200
GGACGGCAAC AAGTTTGCCA AGCTCATAGT GGAGACGGAC ACGTTTGGCA 250
GCCGGGTTCG CATCAAAGGG GCTGAGAGTG AGAAGTACAT CTGTATGAAC 300
AAGAGGGGCA AGCTCATCGG GAAGCCCAGC GGGAAGAGCA AAGACTGCGT 350
GTTCACGGAG ATCGTGCTGG AGAACAACTA TACGGCCTTC CAGAACGCCC 400
GGCACGAGGG CTGGTTCATG GCCTTCACGC GGCAGGGGCG GCCCCGCCAG 450
GCTTCCCGCA GCCGCCAGAA CCAGCGCGAG GCCCACTTCA TCAAGCGCCT 500
CTACCAAGGC CAGCTGCCCT TCCCCAACCA CGCCGAGAAG CAGAAGCAGT 550
TCGAGTTTGT GGGCTCCGCC CCCACCCGCC GGACCAAGCG CACACGGCGG 600
CCCCAGCCCC TCACGTAGTC TGGGAGGCAG GGGGCAGCAG CCCCTGGGCC 650
GCCTCCCCAC CCCTTTCCCT TCTTAATCCA AGGACTGGGC TGGGGTGGCG 700
GGAGGGGAGC CAGATCCCCG AGGGAGGACC CTGAGGGCCG CGAAGCATCC 750
GAGCCCCCAG CTGGGAAGGG GCAGGCCGGT GCCCCAGGGG CGGCTGGCAC 800
AGTGCCCCCT TCCCGGACGG GTGGCAGGCC CTGGAGAGGA ACTGAGTGTC 850
ACCCTGATCT CAGGCCACCA GCCTTTGCCG GCCTCCCAGC CGGGCTCCTG 900
AAGCCCGCTG AAAGGTCAGC GACTGAAGGC CTTGCAGACA ACCGTCTGGA 950
GGTGGCTGTC CTCAAAATCT GCTTCTCGGA TCTCCCTCAG TCTGCCCCCA 1000
GCCCCCAAAC TCCTCCTGGC TAGACTGTAG GAAGGGACTT TTGTTTGTTT 1050
GTTTGTTTCA GGAAAAAAGA AAGGGAGAGA GAGGAAAATA GAGGGTTGTC 1100
CACTCCTCAC ATTCCACGAC CCAGGCCTGC ACCCCACCCC CAACTCCCAG 1150
CCCCGGAATA AAACCATTTT CCTGC 1175
```

FIG. 1

DNA27864

```
Met Gly Ala Ala Arg Leu Leu Pro Asn Leu Thr Leu Cys Leu Gln
 1               5                  10                  15

Leu Leu Ile Leu Cys Cys Gln Thr Gln Tyr Val Arg Asp Gln Gly
            20                  25                  30

Ala Met Thr Asp Gln Leu Ser Arg Arg Gln Ile Arg Glu Tyr Gln
        35                  40                  45

Leu Tyr Ser Arg Thr Ser Gly Lys His Val Gln Val Thr Gly Arg
    50                  55                  60

Arg Ile Ser Ala Thr Ala Glu Asp Gly Asn Lys Phe Ala Lys Leu
65                  70                  75

Ile Val Glu Thr Asp Thr Phe Gly Ser Arg Val Arg Ile Lys Gly
            80                  85                  90

Ala Glu Ser Glu Lys Tyr Ile Cys Met Asn Lys Arg Gly Lys Leu
        95                  100                 105

Ile Gly Lys Pro Ser Gly Lys Ser Lys Asp Cys Val Phe Thr Glu
    110                 115                 120

Ile Val Leu Glu Asn Asn Tyr Thr Ala Phe Gln Asn Ala Arg His
125                 130                 135

Glu Gly Trp Phe Met Ala Phe Thr Arg Gln Gly Arg Pro Arg Gln
            140                 145                 150

Ala Ser Arg Ser Arg Gln Asn Gln Arg Glu Ala His Phe Ile Lys
        155                 160                 165

Arg Leu Tyr Gln Gly Gln Leu Pro Phe Pro Asn His Ala Glu Lys
    170                 175                 180

Gln Lys Gln Phe Glu Phe Val Gly Ser Ala Pro Thr Arg Arg Thr
185                 190                 195

Lys Arg Thr Arg Arg Pro Gln Pro Leu Thr
            200                 205
```

FIG. 2

SUBSTITUTE SHEET (RULE 26)

ss.DNA26645 (237 bp)

	Score	Match	Pct
S78462S3 AIGF=androgen-induced growth factor AIGF [human]	310	84	74

S78462S3 AIGF=androgen-induced growth factor AIGF [human, placenta (190 bp)
Score = 310 (85.7 bits), Expect = 7.1e-17, P = 7.1e-17
Identities = 84/113 (74%), Positives = 84/113 (74%), at 73,23, Strand = +/+

```
DNA26645    73 CTAATTTTAACCAGTACGTGAGGGACCAGGGCGCCATGACCGACCAGNTGAGCAGGCGGC
               *********  *** * ******** *** **     **** ** *** * *** * ** *
S78462S3    23 CTAATTTTACACAGCATGTGAGGGAGCAGAGCCTGGTGACGGATCAGCTCAGCCGCCGCC

DNA26645   133 AGATCCGCGAGTACCAANTNTACAGCAGGACCAGTGGCAAGCACGTGCAGGTC
                 *****    ****** * ****** * ***** ** ***************
S78462S3    83 TCATCCGGACCTACCAACTCTACAGCCGCACCAGCGGGAAGCACGTGCAGGTC
```

FIG. 3

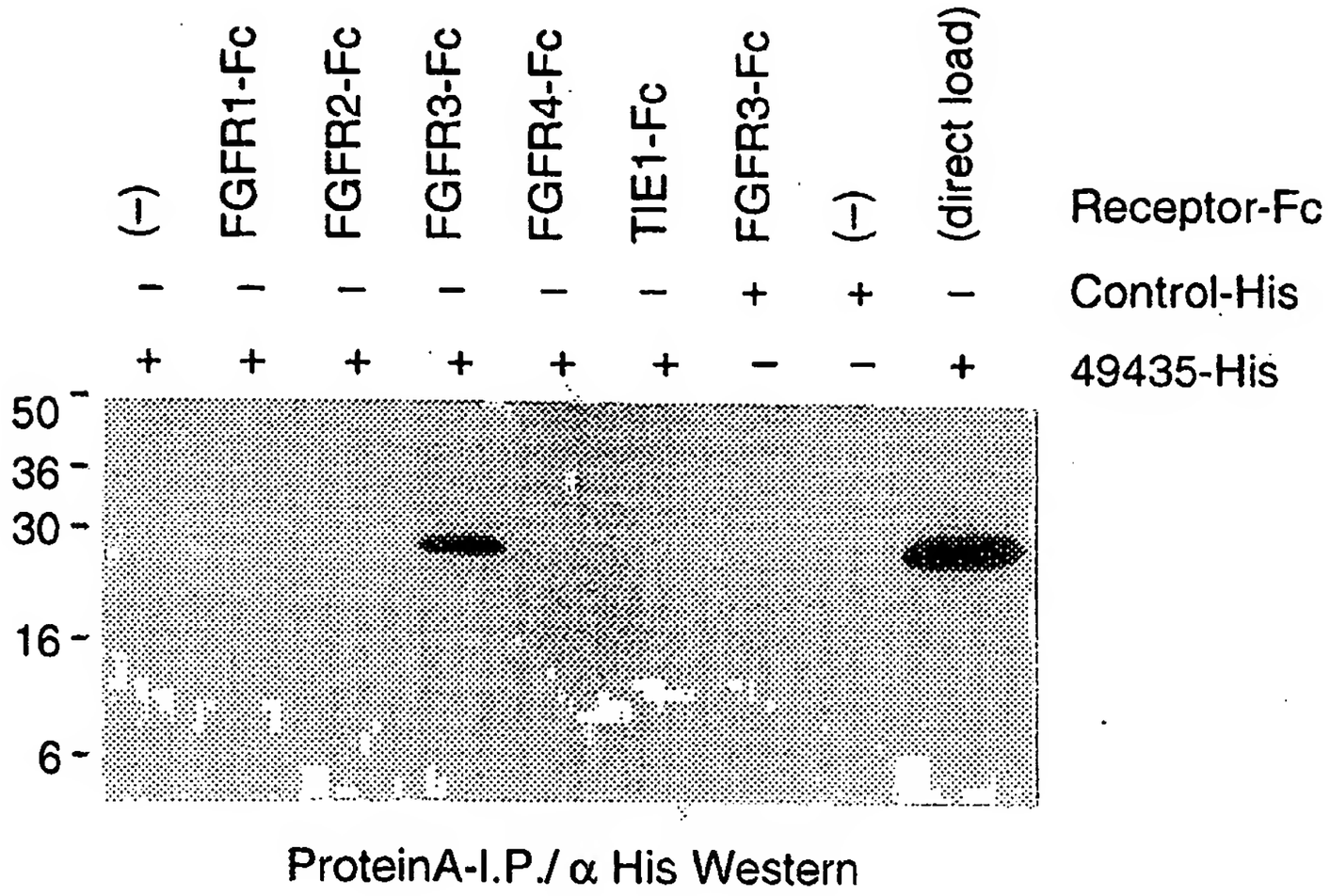
(–)
FGFR1-Fc
FGFR2-Fc
FGFR3-Fc
FGFR4-Fc
TIE1-Fc
FGFR3-Fc
(–)
(direct load)
Receptor-Fc
– – – – – – + + –
Control-His
+ + + + + + – – +
49435-His
50
36
30
16
6
ProteinA-I.P./ α His Western

FIG. 4

```
CTCCCAGCCA AGAACCTCGG GGCCGCTGCG CGGTGGGGAG GAGTTCCCCG AAACCCGGCC GCTAAGCGAG GCCTCCTCCT CCCGCAGATC CGAACGGCCT 100
GGGCGGGGTC ACCCCGGCTG GGACAAGAAG CCGCCGCCTG CCTGCCCGGG CCCGGGGAGG GGGCTGGGGC TGGGGCCGGA GGCGGGGTGT GAGTGGGTGT 200
GTGCGGGGGG CGGAGGCTTG ATGCAGTCCC GATAAGAAAT GCTCGGGTGT CTTGGGCACC TACCCGTGGG GGCCGTAAGG CGCTACTATA TAACGCTGCC 300
GGCCCTGAGC CGCCGAGCCG TCCGAGCAGG AGCGCTGCGT CCAGGATCTA GGGCACGACC ATCCCAACCC GGCATTCACA GCCCCGCAGC GCATCCGGTC 400
GCCGGCCAGC TTCCGCACCC CATCGCCGGA GCTGCGCCGA GAGCCCCAGG GAGGTGCCAT GCGGAGCGGG TGTGTGGTGG TCCACGTATG GATCCTGGCC 500
GGCCTCTGGC TGGCCGTGGC CGGGCGCCCC CTCGCCTTCT CGGACGCGGG GCCCCACGTG CACTACGGCT GGGGCGACCC CATCCGCCTG CGGCACCTGT 600
ACACCTCCGG CCCCCACGGG CTCTCCAGCT GCTTCCTGCG CATCCGTGCC GACGGCGTCG TGGACTGCGC GCGGGGCCAG AGCGCGCACA GTTTGCTGGA 700
GATCAAGGCA GTCGCTCTGC GGACCGTGGC CATCAAGGGC GTGCACAGCG TGCGGTACCT CTGCATGGGC GCCGACGGCA AGATGCAGGG GCTGCTTCAG 800
TACTCGGAGG AAGACTGTGC TTTCGAGGAG GAGATCCGCC CAGATGGCTA CAATGTGTAC CGATCCGAGA AGCACCGCCT CCCGGTCTCC CTGAGCAGTG 900
CCAAACAGCG GCAGCTGTAC AAGAACAGAG GCTTTCTTCC ACTCTCTCAT TTCCTGCCCA TGCTGCCCAT GGTCCCAGAG GAGCCTGAGG ACCTCAGGGG 1000
CCACTTGGAA TCTGACATGT TCTCTTCGCC CCTGGAGACC GACAGCATGG ACCCATTTGG GCTTGTCACC GGACTGGAGG CCGTGAGGAG TCCCAGCTTT 1100
GAGAAGTAAC TGAGACCATG CCCGGGCCTC TTCACTGCTG CCAGGGGCTG TGGTACCTGC AGCGTGGGGG ACGTGCTTCT ACAAGAACAG TCCTGAGTCC 1200
ACGTTCTGTT TAGCTTTAGG AAGAAACATC TAGAAGTTGT ACATATTCAG AGTTTTCCAT TGGCAGTGCC AGTTTCTAGC CAATAGACTT GTCTGATCAT 1300
AACATTGGAA GCCTTGTACT TGGCCCAGCT GTTGCCTGGG CCCCCCATTC TGCTCCCTCG AGGTTGCTGG ACAAGCTGCT GCACTGTCTC AGTTCTGCTT 1400
GAATACCTCC ATCGATGGGG AACTCACTTC CTTTGGAAAA ATTCTTATGT CAAGCTGAAA TTCTCTAATT TTTCTCATCA CTTCCCCAGG AGCAGCCAGA 1500
AGACAGGCAG TAGTTTTAAT TTCAGGAACA GGTGATCCAC TCTGTAAAAC AGCAGGTAAA TTTCACTCAA CCCCATGTGG GAATTGATCT ATATCTCTAC 1600
TTCCAGGGAC CATTTGCCCT TCCCAAATCC CTCCAGGCCA GAACTGACTG GAGCAGGCAT GGCCCACCAG GCTTCAGAAG TAGGGGAAGC CTGGAGCCCC 1700
ACTCCAGCCC TGGGACAACT TGAGAATTCC CCCTGAGGCC AGTTCTGTCA TGGATGCTGT CCTGAGAATA ACTTGCTGTC CCGGTGTCAC CTGCTTCCAT 1800
CTCCCAGCCC ACCAGCCCTC TGCCCACCTC ACATGCCTCC CCATGGATTG GGGCCTCCCA GGCCCCCCAC CTTATGTCAA CCTGCACTTC TTGTTCAAAA 1900
ATCAGGAAAA GAAAAGATTT GAAGACCCCA AGTCTTGTCA ATAACTTGCT GTGTGGAAGC AGCGGGGGAA GACCTAGAAC CCTTTCCCCA GCACTTGGTT 2000
TTCCAACATG ATATTTATGA GTAATTTATT TTGATATGTA CATCTCTTAT TTTCTTACAT TATTTATGCC CCCAAATTAT ATTTATGTAT GTAAGTGAGG 2100
TTTGTTTTGT ATATTAAAAT GGAGTTTGTT TGT 2133
```

FIG. 5

```
Met Arg Ser Gly Cys Val Val Val His Val Trp Ile Leu Ala Gly
 1               5                  10                  15

Leu Trp Leu Ala Val Ala Gly Arg Pro Leu Ala Phe Ser Asp Ala
            20                  25                  30

Gly Pro His Val His Tyr Gly Trp Gly Asp Pro Ile Arg Leu Arg
        35                  40                  45

His Leu Tyr Thr Ser Gly Pro His Gly Leu Ser Ser Cys Phe Leu
    50                  55                  60

Arg Ile Arg Ala Asp Gly Val Val Asp Cys Ala Arg Gly Gln Ser
65                  70                  75

Ala His Ser Leu Leu Glu Ile Lys Ala Val Ala Leu Arg Thr Val
            80                  85                  90

Ala Ile Lys Gly Val His Ser Val Arg Tyr Leu Cys Met Gly Ala
        95                  100                 105

Asp Gly Lys Met Gln Gly Leu Leu Gln Tyr Ser Glu Glu Asp Cys
    110                 115                 120

Ala Phe Glu Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Arg
125                 130                 135

Ser Glu Lys His Arg Leu Pro Val Ser Leu Ser Ser Ala Lys Gln
            140                 145                 150

Arg Gln Leu Tyr Lys Asn Arg Gly Phe Leu Pro Leu Ser His Phe
        155                 160                 165

Leu Pro Met Leu Pro Met Val Pro Glu Glu Pro Glu Asp Leu Arg
    170                 175                 180

Gly His Leu Glu Ser Asp Met Phe Ser Ser Pro Leu Glu Thr Asp
185                 190                 195

Ser Met Asp Pro Phe Gly Leu Val Thr Gly Leu Glu Ala Val Arg
            200                 205                 210

Ser Pro Ser Phe Glu Lys
        215 216
```

FIG. 6

SUBSTITUTE SHEET (RULE 26)

	Frame	Score	Match	Pct
AF007268_1 fibroblast growth factor - Mus musculus	+3	509	113	53

AF007268_1 fibroblast growth factor - Mus musculus (218 aa)
Score = 509 (179.2 bits), Expect = 5.1e-48, P = 5.1e-48
Identities = 113/214 (52%), Positives = 147/214 (68%), at 465,6, Frame = +3

```
DNA49435   465 SGCVVVHVWILAGLWLAVAGRPLA-----FSDAGPHVHYGWGDPIRLRHLYTSGPHGLSS
               .*  *    .** *****.*****      **  *   ****   **..**..**. .*.
AF007268_1   6 NGRAVARALVLATLWLAVSGRPLAQQSQSVSDEDPLFLYGWGKITRLQYLYSAGPY-VSN

DNA49435   630 CFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSE
               ******.** ***   *.  .*** .****.*.*** * ******* ****. **..***
AF007268_1  65 CFLRIRSDGSVDCEEDQNERNLLEFRAVALKTIAIKDVSSVRYLCMSADGKIYGLIRYSE

DNA49435   810 EDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPE
               *** * **.   *** *** ** * .    ** *.  ...   * *.*.*.      *
AF007268_1 125 EDCTFREEMDCLGYNQYRSMKHHLHIIFIQAKPREQLQDQK--P-SNFIPVFHRSFFETG

DNA49435   990 DLRGHLESDMFSSPLETDSMDPFGLVTGLE-AVRSPSFEK
               *    * * *** ***.****** .*  ..  *.****.*
AF007268_1 182 D---QLRSKMFSLPLESDSMDPFRMVEDVDHLVKSPSFQK
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FIG. 7

```
CTGTCGGAGC AGTAACTCTG TGCGCCCCAC GCCACAAGCG CCCAGTTGCT TTGTGGGTTG TGCCTGCCCT GCGCCTGCAA CTTGAGTCCC CGCCGCATCG 100
CAGTCTCCGC GCCACCTTTG TAACGGCCTT CAGGACCCCG AGGTGTCATG GCGAGAAAGT GGAACGGGCG TGCGGTGGCC CGAGCCCTGG TCCTGGCCAC 200
TCTGTGGCTG GCTGTGTCTG GGCGTCCCCT GGCCCAGCAA TCCCAGTCTG TGTCAGATGA AGATCCACTC TTTCTCTACG GCTGGGGCAA GATTACCCGC 300
CTGCAGTACC TGTACTCCGC TGGTCCCTAT GTCTCCAACT GCTTCCTCCG AATCCGGAGC GACGGCTCTG TGGACTGCGA GGAGGACCAA AACGAACGAA 400
ATTTGTTGGA ATTCCGCGCG GTCGCTCTGA AGACGATTGC CATCAAGGAC GTCAGCAGCG TGCGGTACCT CTGCATGAGC GCGGACGGCA AGATATACGG 500
GCTGATTCGC TACTCGGAGG AAGACTGTAC CTTCAGGGAG GAAATGGACT GTTTAGGCTA CAACCAGTAC AGATCCATGA AGCACCATCT CCATATCATC 600
TTCATCCAGG CCAAGCCCAG AGAACAGCTC CAGGACCAGA AACCCTCAAA CTTTATCCCC GTGTTTCACC GCTCCTTCTT TGAAACCGGG GACCAGCTGA 700
GGTCTAAAAT GTTCTCCCTG CCCCTGGAGA GTGACAGCAT GGATCCGTTC AGGATGGTGG AGGATGTAGA CCACCTAGTG AAGAGTCCCA GCTTCCAGAA 800
ATGACAGGAT TCCGACAGGA TGGAGAAAAC CCCAAGGTCC CGTGAACTTC CCCCTTAGGA AGCTGTAGAT ATTCTAAGTC TCACATGGAC CCTGTTGTGT 900
TAGTGGCTAG ACTTGATCAT GAACCTAAGT TGACAACCTG CCTGGCTGCC ATCGGAGCCC CACTGACTTT GGAGGCTGCT GATATGTGCC TAAGTTACTC 1000
CAGTTCTGTT TGAATACCTC CACTAATAGG GAACTTACTC CTGTGAAACA TTCTTAGTTT TGAGCCAAAT CTGTGACTTG GATGGTTTTA GCGAGCAAGC 1100
CAGAAGGTAT GAAGTCAAAT GATAAAATTC ATGTATAGAA AGTGGGCTCT AAAATATATA TTCCCTATAT GGATCTCATG GGATCTTAGC TTGCCCCCCA 1200
AATGTCTCCT GGCCAGAACT AACTGGGGTT ACAAACTTGG AACAAAGGAC AGCCTAGAAA ACTTTGGGAG CCTTGAAGGA TGGTCTTAGG ATTACGAATT 1300
CCAGCTGACT ACGTAGCTTC CCCCTTTTCC ACTTATAAAT GTCAGATGGA AGTGACCCTT AGCTGAGTGC ATAGCCAAGC TGCCACTTAT GCCCCAGGAG 1400
CTTGTCTCTG TCCCATGACC CCAGATTTCC AGGACCTGGA TCTGCTCCTC TGACCTTTCC CAGAGTTCAC CTGGGCTCTC CAACCCCAGA GCAGGTAGCT 1500
TATGAGCCAT CCAGTTGTGT CCCCAGCTCC TGGCTCTCAG TTCTGGTCAC CAAACATTGT GAATCAACGT GTCTGCTGCC TGTGTCAACC TGGATCCCTC 1600
ATTTACAAAC AAGATTAGGA AGCCCCAAAT TCTCCAGTGG CAGCTGGGGA ACTGTGGAGT CCTTTCCCCG GCACTTACGT GGCAGCATGA TATTTATAAG 1700
TAATTTATTG TGTGTGTGTC TTCTATTTTC TTACTTTATT TATGCCCCAG ATCATATTTA TGTACATGAC TTGTTTTCTA CATTAAAAAG GAGTTGGTTT 1800
GTATCAAAAA AAAAAAAAAA AAAA 1824
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FIG. 8

SUBSTITUTE SHEET (RULE 26)

ss.DNA32286

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CGGACGCGTG GGCGGACGCG TGGGCGGCCC ACGGCGCCCG CGGGCTGGGG CGGTCGCTTC TTCCTTCTCC GTGGCCTACG AGGGTCCCCA GCCTGGGTAA 100
AGATGGCCCC ATGGCCCCCG AAGGGCCTAG TCCCAGCTGT GCTCTGGGGC CTCAGCCTCT TCCTCAACCT CCCAGGACCT ATCTGGCTCC AGCCCTCTCC 200
ACCTCCCCAG TCTTCTCCCC CGCCTCAGCC CCATCCGTGT CATACCTGCC GGGGACTGGT TGACAGCTTT AACAAGGGCC TGGAGAGAAC CATCCGGGAC 300
AACTTTGGAG GTGGAAACAC TGCCTGGGAG GAAGAGAATT TGTCCAAATA CAAAGACAGT GAGACCCGCC TGGTAGAGGT GCTGGAGGGT GTGTGCAGCA 400
AGTCAGACTT CGAGTGCCAC CGCCTGCTGG AGCTGAGTGA GGAGCTGGTG GAGAGCTGGT GGTTTCACAA GCAGCAGGAG GCCCCGGACC TCTTCCAGTG 500
GCTGTGCTCA GATTCCCTGA AGCTCTGCTG CCCCGCAGGC ACCTTCGGGC CCTCCTGCCT TCCCTGTCCT GGGGGAACAG AGAGGCCCTG CGGTGGCTAC 600
GGGCAGTGTG AAGGAGAAGG GACACGAGGG GGCAGCGGGC ACTGTGACTG CCAAGCCGGC TACGGGGGTG AGGCCTGTGG CCAGTGTGGC CTTGGCTACT 700
TTGAGGCAGA ACGCAACGCC AGCCATCTGG TATGTTCGGC TTGTTTTGGC CCCTGTGCCC GATGCTCAGG ACCTGAGGAA TCAAACTGTT TGCAATGCAA 800
GAAGGGCTGG GCCCTGCATC ACCTCAAGTG TGTAGACATT GATGAGTGTG GCACAGAGGG AGCCAACTGT GGAGCTGACC AATTCTGCGT GAACACTGAG 900
GGCTCCTATG AGTGCCGAGA CTGTGCCAAG GCCTGCCTAG GCTGCATGGG GGCAGGGCCA GGTCGCTGTA AGAAGTGTAG CCCTGGCTAT CAGCAGGTGG 1000
GCTCCAAGTG TCTCGATGTG GATGAGTGTG AGACAGAGGT GTGTCCGGGA GAGAACAAGC AGTGTGAAAA CACCGAGGGC GGTTATCGCT GCATCTGTGC 1100
CGAGGGCTAC AAGCAGATGG AAGGCATCTG TGTGAAGGAG CAGATCCCAG AGTCAGCAGG CTTCTTCTCA GAGATGACAG AAGACGAGTT GGTGGTGCTG 1200
CAGCAGATGT TCTTTGGCAT CATCATCTGT GCACTGGCCA CGCTGGCTGC TAAGGGCGAC TTGGTGTTCA CCGCCATCTT CATTGGGGCT GTGGCGGCCA 1300
TGACTGGCTA CTGGTTGTCA GAGCGCAGTG ACCGTGTGCT GGAGGGCTTC ATCAAGGGCA GATAATCGCG GCCACCACCT GTAGGACCTC CTCCCACCCA 1400
CGCTGCCCCC AGAGCTTGGG CTGCCCTCCT GCTGGACACT CAGGACAGCT TGGTTTATTT TTGAGAGTGG GGTAAGCACC CCTACCTGCC TTACAGAGCA 1500
GCCCAGGTAC CCAGGCCCGG GCAGACAAGG CCCCTGGGGT AAAAAGTAGC CCTGAAGGTG GATACCATGA GCTCTTCACC TGGCGGGGAC TGGCAGGCTT 1600
CACAATGTGT GAATTTCAAA AGTTTTTCCT TAATGGTGGC TGCTAGAGCT TTGGCCCCTG CTTAGGATTA GGTGGTCCTC ACAGGGGTGG GGCCATCACA 1700
GCTCCCTCCT GCCAGCTGCA TGCTGCCAGT TCCTGTTCTG TGTTCACCAC ATCCCCACAC CCCATTGCCA CTTATTTATT CATCTCAGGA AATAAAGAAA 1800
GGTCTTGGAA AGTTAAAAAA AAAAAAAAAA AAAAAAAA 1838
```

FIG. 9

P1.DNA32286

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Met Ala Pro Trp Pro Pro Lys Gly Leu Val Pro Ala Val Leu Trp Gly Leu Ser Leu Phe Leu Asn Leu Pro Gly Pro Ile Trp Leu Gln
 1               5                  10                  15                  20                  25                  30

Pro Ser Pro Pro Pro Gln Ser Ser Pro Pro Pro Gln Pro His Pro Cys His Thr Cys Arg Gly Leu Val Asp Ser Phe Asn Lys Gly Leu
            35                  40                  45                  50                  55                  60

Glu Arg Thr Ile Arg Asp Asn Phe Gly Gly Gly Asn Thr Ala Trp Glu Glu Glu Asn Leu Ser Lys Tyr Lys Asp Ser Glu Thr Arg Leu
        65                  70                  75                  80                  85                  90

Val Glu Val Leu Glu Gly Val Cys Ser Lys Ser Asp Phe Glu Cys His Arg Leu Leu Glu Leu Ser Glu Glu Leu Val Glu Ser Trp Trp
                95                 100                 105                 110                 115                 120

Phe His Lys Gln Gln Glu Ala Pro Asp Leu Phe Gln Trp Leu Cys Ser Asp Ser Leu Lys Leu Cys Cys Pro Ala Gly Thr Phe Gly Pro
           125                 130                 135                 140                 145                 150

Ser Cys Leu Pro Cys Pro Gly Gly Thr Glu Arg Pro Cys Gly Gly Tyr Gly Gln Cys Glu Gly Glu Gly Thr Arg Gly Gly Ser Gly His
       155                 160                 165                 170                 175                 180

Cys Asp Cys Gln Ala Gly Tyr Gly Gly Glu Ala Cys Gly Gln Cys Gly Leu Gly Tyr Phe Glu Ala Glu Arg Asn Ala Ser His Leu Val
                185                 190                 195                 200                 205                 210

Cys Ser Ala Cys Phe Gly Pro Cys Ala Arg Cys Ser Gly Pro Glu Glu Ser Asn Cys Leu Gln Cys Lys Lys Gly Trp Ala Leu His His
           215                 220                 225                 230                 235                 240

Leu Lys Cys Val Asp Ile Asp Glu Cys Gly Thr Glu Gly Ala Asn Cys Gly Ala Asp Gln Phe Cys Val Asn Thr Glu Gly Ser Tyr Glu
           245                 250                 255                 260                 265                 270

Cys Arg Asp Cys Ala Lys Ala Cys Leu Gly Cys Met Gly Ala Gly Pro Gly Arg Cys Lys Lys Cys Ser Pro Gly Tyr Gln Gln Val Gly
           275                 280                 285                 290                 295                 300

Ser Lys Cys Leu Asp Val Asp Glu Cys Glu Thr Glu Val Cys Pro Gly Glu Asn Lys Gln Cys Glu Asn Thr Glu Gly Gly Tyr Arg Cys
       305                 310                 315                 320                 325                 330

Ile Cys Ala Glu Gly Tyr Lys Gln Met Glu Gly Ile Cys Val Lys Glu Gln Ile Pro Glu Ser Ala Gly Phe Phe Ser Glu Met Thr Glu
           335                 340                 345                 350                 355                 360

Asp Glu Leu Val Val Leu Gln Gln Met Phe Phe Gly Ile Ile Ile Cys Ala Leu Ala Thr Leu Ala Ala Lys Gly Asp Leu Val Phe Thr
           365                 370                 375                 380                 385                 390

Ala Ile Phe Ile Gly Ala Val Ala Ala Met Thr Gly Tyr Trp Leu Ser Glu Arg Ser Asp Arg Val Leu Glu Gly Phe Ile Lys Gly Arg
           395                 400                 405                 410                 415                 420
```

FIG. 10

SUBSTITUTE SHEET (RULE 26)

GGGAACGGAAAATGGCGCCTCACGGCCCGGGTAGTCTTACGACCCTGGTGCCCTGGGCTGCCGCC
CTGCTCCTCGCTCTGGGCGTGGAAAGGGCTCTGGCGCTACCCGAGATATGCACCCAATGTCCAGG
GAGCGTGCAAAATTTGTCAAAAGTGGCCTTTTATTGTAAAACGACACNAGAGCTAATGCTGCATG
CCCNTTGCTGCCTGAATCAGAAGGGCACCATCTTGGGGCTGGATCTCCAGAACTGTTCTCTGGAG
GACCCTGGTCCAAACTTTCATCAGGCACATACCACTGTCATCATAGACCTGCAAGCAAACCCCCT
CAAAGGTGACTTGGCCAACACCTTCCGTGGCTTTACTCAGCTCCAGACTCTGATACTGCCACAAC
ATGTCAACTGTCCTGGAGGAATTAATGCCTGGAATACTATCACCTCTTATATAGACAACCAAATC
TGTCAAGGGCAAAAGAACCTTTGCAATAACACTGGGGACCCAGAAATGTGTCCTGAGAATGGATC
TTGTGTACCTGATGGTCCAGGTCTTTTGCAGTGTGTTTGTGCTGATGGTTTCCATGGATACAAGT
GTATGCGCCAGGGCTCGTTCTCACTGCTTATGTTCTTCGGGATTCTGGGAGCCACCACTCTATCC
GTCTCCATTCTGCTTTGGGCGACCCAGCGCCGAAAAGCCAAGACTTCATGAACTACATAGGTCTT
ACCATTGACCTAAGATCAATCTGAACTATCTTAGCCCAGTCAGGGAGCTCTGCTTCCTAGAAAGG
CATCTTTCGCCAGTGGATTCGCCTCAAGGTTGAGGCCGCCATTGGAAGATGAAAAATTGCACTCC
CTTGGTGTAGACAAATACCAGTTCCCATTGGTGTTGTTGCCTATAATAAACACTTTTTCTTTTTT
NAAAAAAAAAAAAAAAAAAAAA

FIG. 11

MAPHGPGSLTTLVPWAAALLLALGVERALALPEICTQCPGSVQNLSKVAFYCKTTXELML
HAXCCLNQKGTILGLDLQNCSLEDPGPNFHQAHTTVIIDLQANPLKGDLANTFRGFTQLQ
TLILPQHVNCPGGINAWNTITSYIDNQICQGQKNLCNNTGDPEMCPENGSCVPDGPGLLQ
CVCADGFHGYKCMRQGSFSLLMFFGILGATTLSVSILLWATQRRKAKTS

FIG. 12

><DNA30873: 15 members (6 incyte, 9 est) 579 bp, 20 gaps, 354 bp
orf (+3)

ACACCTTCCGTGGCTTTACTCAGCTCCAGACTCTGATACTGCCACAACAT
GTCAACTGTCCTGGAGGAATTAATGCCTGGAATACTATCACCTCTTATAT
AGACAACCAAATCTGTCAAGGGCAAAAGAACCTTTGCAATAACACTGGGG
ACCCAGAAATGTGTCCTGAGAATGGATCTTGTGTACCTGATGGTCCAGGT
CTTTTGCAGTGTGTTTGTGCTGATGGTTTCCATGGATACAAGTGTATGCG
CCAGGG CTC GTTCTCACTGCTTATGTTCTTC GGGATTCTGGGATCCA
CCACTCTATCCGTCTCCATTCTGCTTT GGGCGACCCAGCGCCG AAAAG
CCAAGACTT CATGAACTACATAGGTCTTACCATTG ACCTAAGATCAAT
CTG AACTATCTTAG CCC AGTCAGGG AGCTCTGCCTCCTAG AAAG
GCATCTTT CGCC AGTGG ATT CGCCTCAA GGTTGA GGCCGCCATT
GG AAGATGAAAAATTGCACTCCCTTGGTGTAGACAAATACCAGTTCCCA
TTGGGTGTTGGTTGCCTATAATAAACACTTTTTTCTTTTTTAAAAAAAA

FIG. 13

SUBSTITUTE SHEET (RULE 26)

```
  DNA34387   113 FRGFTQLQTLILP--QHVNCPGGINAWNTI--TSYIDNQICQGQKNLCNNTGDPEMCPEN
               .**    . .. *  .* .* *  .**  .  *..    .*    * *   *  * *
SERR-DROME   311 WRGPLCNECMVYPGCKHGSCNG--SAWKCVCDTNW-GGILCDQDLNFC---GTHEPCKHG

  DNA34387   311 GSCVPDGPGLLQCVCADGFHGYKC
               *.*    *   .* **.*  * .*
SERR-DROME   365 GTCENTAPDKYRCTCAEGLSGEQC
```

FIG. 14

```
   DNA34387   127 HVNCPGG--INAWNTI--TSYIDNQICQGQKNLCNNTGDPEMCPENGSCVPDGPGLLQCV
                * * * * * . *.. *.* * * * * * *.* ** **
GGCSERRAT_1   242 HPGCVHGTCIEPWQCLCETNW-GGQLCDKDLNYCG-THPP--CLNGGTCSNTGPDKYQCS

   DNA34387   183 CADGFHGYKC
                * .*. * *
GGCSERRAT_1   298 CPEGYSGQNC
```

FIG. 15

SUBSTITUTE SHEET (RULE 26)

ss.DNA32292

```
GGCCGGAGCA GCACGGCCGC AGGACCTGGA GCTCCGGCTG CGTCTTCCCG CAGCGCTACC CGCCATGCGC CTGCCGCGCC GGGCCGCGCT GGGGCTCCTG 100
CCGCTTCTGC TGCTGCTGCC GCCCGCGCCG GAGGCCGCCA AGAAGCCGAC GCCCTGCCAC CGGTGCCGGG GGCTGGTGGA CAAGTTTAAC CAGGGGATGG 200
TGGACACCGC AAAGAAGAAC TTTGGCGGCG GGAACACGGC TTGGGAGGAA AAGACGCTGT CCAAGTACGA GTCCAGCGAG ATTCGCCTGC TGGAGATCCT 300
GGAGGGGCTG TGCGAGAGCA GCGACTTCGA ATGCAATCAG ATGCTAGAGG CGCAGGAGGA GCACCTGGAG GCCTGGTGGC TGCAGCTGAA GAGCGAATAT 400
CCTGACTTAT TCGAGTGGTT TTGTGTGAAG ACACTGAAAG TGTGCTGCTC TCCAGGAACC TACGGTCCCG ACTGTCTCGC ATGCCAGGGC GGATCCCAGA 500
GGCCCTGCAG CGGGAATGGC CACTGCAGCG GAGATGGGAG CAGACAGGGC GACGGGTCCT GCCGGTGCCA CATGGGGTAC CAGGGCCCGC TGTGCACTGA 600
CTGCATGGAC GGCTACTTCA GCTCGCTCCG GAACGAGACC CACAGCATCT GCACAGCCTG TGACGAGTCC TGCAAGACGT GCTCGGGCCT GACCAACAGA 700
GACTGCGGCG AGTGTGAAGT GGGCTGGGTG CTGGACGAGG GCGCCTGTGT GGATGTGGAC GAGTGTGCGG CCGAGCCGCC TCCCTGCAGC GCTGCGCAGT 800
TCTGTAAGAA CGCCAACGGC TCCTACACGT GCGAAGAGTG TGACTCCAGC TGTGTGGGCT GCACAGGGGA AGGCCCAGGA AACTGTAAAG AGTGTATCTC 900
TGGCTACGCG AGGGAGCACG GACAGTGTGC AGATGTGGAC GAGTGCTCAC TAGCAGAAAA AACCTGTGTG AGGAAAAACG AAAACTGCTA CAATACTCCA 1000
GGGAGCTACG TCTGTGTGTG TCCTGACGGC TTCGAAGAAA CGGAAGATGC CTGTGTGCCG CCGGCAGAGG CTGAAGCCAC AGAAGGAGAA AGCCCGACAC 1100
AGCTGCCCTC CCGCGAAGAC CTGTAATGTG CCGGACTTAC CCTTTAAATT ATTCAGAAGG ATGTCCCGTG GAAAATGTGG CCCTGAGGAT GCCGTCTCCT 1200
GCAGTGGACA GCGGCGGGGA GAGGCTGCCT GCTCTCTAAC GGTTGATTCT CATTTGTCCC TTAAACAGCT GCATTTCTTG GTTGTTCTTA AACAGACTTG 1300
TATATTTTGA TACAGTTCTT TGTAATAAAA TTGACCATTG TAGGTAATCA GGAGGAAAAA AAAA 1364
```

FIG. 16

P1.DNA 32292

```
Met Arg Leu Pro Arg Arg Ala Ala Leu Gly Leu Leu Pro Leu Leu Leu Leu Leu Pro Pro Ala Pro Glu Ala Ala Lys Lys Pro Thr Pro
1               5                  10                  15                  20                  25                  30

Cys His Arg Cys Arg Gly Leu Val Asp Lys Phe Asn Gln Gly Met Val Asp Thr Ala Lys Lys Asn Phe Gly Gly Gly Asn Thr Ala Trp
            35                  40                  45                  50                  55                  60

Glu Glu Lys Thr Leu Ser Lys Tyr Glu Ser Ser Glu Ile Arg Leu Leu Glu Ile Leu Glu Gly Leu Cys Glu Ser Ser Asp Phe Glu Cys
        65                  70                  75                  80                  85                  90

Asn Gln Met Leu Glu Ala Gln Glu Glu His Leu Glu Ala Trp Trp Leu Gln Leu Lys Ser Glu Tyr Pro Asp Leu Phe Glu Trp Phe Cys
                95                 100                 105                 110                 115                 120

Val Lys Thr Leu Lys Val Cys Cys Ser Pro Gly Thr Tyr Gly Pro Asp Cys Leu Ala Cys Gln Gly Gly Ser Gln Arg Pro Cys Ser Gly
            125                 130                 135                 140                 145                 150

Asn Gly His Cys Ser Gly Asp Gly Ser Arg Gln Gly Asp Gly Ser Cys Arg Cys His Met Gly Tyr Gln Gly Pro Leu Cys Thr Asp Cys
        155                 160                 165                 170                 175                 180

Met Asp Gly Tyr Phe Ser Ser Leu Arg Asn Glu Thr His Ser Ile Cys Thr Ala Cys Asp Glu Ser Cys Lys Thr Cys Ser Gly Leu Thr
                185                 190                 195                 200                 205                 210

Asn Arg Asp Cys Gly Glu Cys Glu Val Gly Trp Val Leu Asp Glu Gly Ala Cys Val Asp Val Asp Glu Cys Ala Ala Glu Pro Pro Pro
            215                 220                 225                 230                 235                 240

Cys Ser Ala Ala Gln Phe Cys Lys Asn Ala Asn Gly Ser Tyr Thr Cys Glu Glu Cys Asp Ser Ser Cys Val Gly Cys Thr Gly Glu Gly
        245                 250                 255                 260                 265                 270

Pro Gly Asn Cys Lys Glu Cys Ile Ser Gly Tyr Ala Arg Glu His Gly Gln Cys Ala Asp Val Asp Glu Cys Ser Leu Ala Glu Lys Thr
            275                 280                 285                 290                 295                 300

Cys Val Arg Lys Asn Glu Asn Cys Tyr Asn Thr Pro Gly Ser Tyr Val Cys Val Cys Pro Asp Gly Phe Glu Glu Thr Glu Asp Ala Cys
        305                 310                 315                 320                 325                 330

Val Pro Pro Ala Glu Ala Glu Ala Thr Glu Gly Glu Ser Pro Thr Gln Leu Pro Ser Arg Glu Asp Leu
            335                 340                 345                 350         353
```

FIG. 17

SUBSTITUTE SHEET (RULE 26)

MWRCPLGLLLLLPLAGHLALGAQQGRGRRELAPGLHLRGIRDAGGRYCQEQDLCCRGRAD
DCALPYLGAICYCDLFCNRTVSDCCPDFWDFCLGVPPPFPPIQGCMHGGRIYPVLGTYWD
NCNRCTCQENRQWQCDQEPCLVDPDMIKAINQGNYGWQAGNHSAFWGMTLDEGIRYRLGT
IRPSSSVMNMHEIYTVLNPGEVLPTAFEASEKWPNLIHEPLDQGNCAGSWAFSTAAVASD
RVSIHSLGHMTPVLSPQNLLSCDTHQQQGCRGGRLDGAWWFLRRRGVVSDHCYPFSGRER
DEAGPAPPCMMHSRAMGRGKRQATAHCPNSYVNNNDIYQVTPVYRLGSNDKEIMKELMEN
GPVQALMEVHEDFFLYKGGIYSHTPVSLGRPERYRRHGTHSVKITGWGEETLPDGRTLKY
WTAANSWGPAWGERGHFRIVRGVNECDIESFVLGVWGRVGMEDMGHH

FIG. 18

SUBSTITUTE SHEET (RULE 26)

```
>< Mon Apr 28 22:31:09 1997 [DNA30857], mel
<ORF {trans=1-s, dir=f,res=1}>
CGCCCATCTTCCTCGGTCATGAACATGCATGAAATTTATACAGTGCTGAACCCAGGGGAG
GTGCTTCCCACAGCCTTCGAGGCCTCTGAGAAGTGGCCCAACCTGATTCATGAGCCTCTT
GACCAAGGCAACTGTGCAGGCTCCTGGGCCTTCTCCACAGCAGCTGTGGCATCCGATCGT
GTCTCAATCCATTCTCTGGGACACATGACGCCTGTCCTGTCGCCCCAGAACCTGCTGTCT
TGTGACACCCACCAGCAGCAGGGCTGCCGCGGTGGGCGTCTCGATGGTGCCTGGTGGTTC
CTGCGTCGCCGAGGGGTGGTGTCTGACCACTGCTACCCCTTCTCGGGCCGTGAACGAGAC
GAGGCTGGCCCTGCGCCCCCCTGTATGATGCACAGCCGAGCCATGGGTCGGGGCAAGCGC
CAGGCCACTGCCCACTGCCCCAACAGCTATGTTAATAACAATGACATCTACCAGGTCACT
CCTGTCTACCGCCTCGGCTCCAACGACAAGGAGATCATGAAGGAGCTGATGGAGAATGGC
CCTGTCCAAGCCCTCATGGAGGTGCATGAGGACTTCTTCCTATACAAGGGAGGCATCTAC
AGCCACACGCCAGTGAGCCTTGGGAGGCCAGAGAGATACCGCCGGCATGGGACCCACTCA
GTCAAGATCACAGGATGGGGAGAGGAGACGCTGCCAGATGGAAGGACGCTCAAATACTGG
ACTGCGGCCAACTCCTGGGGCCCAGCCTGGGGCGAGAGGGGCCACTTCCGCATCGTGCGC
GGCGTCAATGAGTGCGACATCGAGAGCTTCGTGCTGGGCGTCTGGGGCCGCGTGGGCATG
GAGGACATGGGTCATCACTGAGGCTNCGGGCACCACGCGGGGTCCGGCCTGGGATCCAGG
CAT
```

FIG. 19

CGGACGCGTGGGCGTCCGGCGGTCGCAGAGCCAGGAGGCGGAGGCGCGCGGGCCAGCCTG
GGCCCCAGCCCACACCTTCACCAGGGCCCAGGAGCCACCATGTGGCGATGTCCACTGGGG
CTACTGCTGTTGCTGCCGCTGGCTGGCCACTTGGCTCTGGGTGCCCAGCAGGGTCGTGGG
CGCCGGGAGCTAGCACCGGGTCTGCACCTGCGGGGCATCCGGGACGCGGGAGGCCGGTAC
TGCCAGGAGCAGGACCTGTGCTGCCGCGGCCGTGCCGACGACTGTGCCCTGCCCTACCTG
GGCGCCATCTGTTACTGTGACCTCTTCTGCAACCGCACGGTCTCCGACTGCTGCCCTGAC
TTCTGGGACTTCTGCCTCGGCGTGCCACCCCCTTTTCCCCCGATCCAAGGATGTATGCAT
GGAGGTCGTATCTATCCAGTCTTGGGAACGTACTGGGACAACTGTAACCGTTGCACCTGC
CAGGAGAACAGGCAGTGGCAGTGTGACCAAGAACCATGCCTGGTGGATCCAGACATGATC
AAAGCCATCAACCAGGGCAACTATGGCTGGCAGGCTGGGAACCACAGCGCCTTCTGGGGC
ATGACCCTGGATGAGGGCATTCGCTACCGCCTGGGCACCATCCGCCCATCTTCCTCGGTC
ATGAACATGCATGAAATTTATACAGTGCTGAACCCAGGGGAGGTGCTTCCCACAGCCTTC
GAGGCCTCTCAGAAGTGGCCCAACCTGATTCATGAGCCTCTTGACCAAGGCAACTGTGCA
GGCTCCTGGGCCTTCTCCACAGCAGCTGTGGCATCCGATCGTGTCTCAATCCATTCTCTG
GGACACATGACGCCTGTCCTGTCGCCCCAGAACCTGCTGTCTTGTGACACCCACCAGCAG
CAGGGCTGCCGCGGTGGGCGTCTCGATGGTGCCTGGTGGTTCCTGCGTCGCCGAGGGGTG
GTGTCTGACCACTGCTACCCCTTCTCGGGCCGTGAACGAGACGAGGCTGGCCCTGCGCCC
CCCTGTATGATGCACAGCCGAGCCATGGGTCGGGGCAAGCGCCAGGCCACTGCCCACTGC
CCCAACAGCTATGTTAATAACAATGACATCTACCAGGTCACTCCTGTCTACCGCCTCGGC
TCCAACGACAAGGAGATCATGAAGGAGCTGATGGAGAATGGCCCTGTCCAAGCCCTCATG
GAGGTGCATGAGGACTTCTTCCTATACAAGGGAGGCATCTACAGCCACACGCCAGTGAGC
CTTGGGAGGCCAGAGLGATACCGCCGGCATGGGACCCACTCAGTCAAGATCACAGGATGG
GGAGAGGAGACGCTGCCAGATGGAAGGACGCTCAAATACTGGACTGCGGCCAACTCCTGG
GGCCCAGCCTGGGGCGAGAGGGGCCACTTCCGCATCGTGCGCGGCGTCAATGAGTGCGAC
ATCGAGAGCTTCGTGCTGGGCGTCTGGGGCCGCGTGGGCATGGAGGACATGGGTCATCAC
TGAGGCTGCGGGCACCACGCGCGGTCCGGCCTGGGATCCAGGCTAAGGGCCGGCGGAAGA
GGCCCCAATGGGGCGGTGACCCCAGCCTCGCCCGACAGAGCCCGGGGCGCAGCCGCGGGC
CAGGGCGCTAATCCCGGCGCGGGTTCCGCTGACGCAGCGCCCCGCCTGGGAGCCGCGGGC
AGGCGAGACTGGCGGAGCCCCCAGACCTCCCAGTGGGGACGGGGCAGGGCCTGGCCTGGG
AAGAGCACAGCTGCAGATCCCAGGCCTCTGGCGCCCCCACTCAAGACTACCAAAGCCAGG
ACACCTCAAGTCTCCAGCCCCAATACCCCACCCCAATCCCGTATTCTTTTTTTTTTTTTTT
TTAGACAGGGTCTTGCTCCGTTGCCCAGGTTGGAGTGCAGTGGCCCATCAGGGCTCACTG
TAACCTCCGACTCCTGGGTTCAAGTGACCCTCCCACCTCAGCCTCTCAAGTAGCTGGGAC
TACAGGTGCACCACCACACCTGGCTAATTTTTGTATTTTTTGTAAAGAGGGGGGTCTCAC
TGTGTTGCCCAGGCTGGTTTCGAACTCCTGGGCTCAAGCGGTCCACCTGCCTCCGCCTCC
CAAAGTGCTGGGATTGCAGGCATGAGCCACTGCACCCAGCCCTGTATTCTTATTCTTCAG
ATATTTATTTTTCTTTTCACTGTTTTAAAATAAAACCAAAGTATTGATAAAAAAAAA

FIG. 20

SUBSTITUTE SHEET (RULE 26)

```
DNA33223    45 GRYCQEQDLCCRGRADDCALPYL--GAICYCDLFCNRTVSDCCPDFWDFCLGVPPPFPPI
               *.**.    ** ** *.*   .    *.**** ** *  ******.  **       *
  A57480    52 GQYCRSLG-CCEGRNDNCVTQFYEANALCYCDKFCERENSDCCPDYKSFCQEEKEWLPHT

DNA33223   103 Q-----GCMHGGRIYPVLGTYWDNCNRCTCQENRQWQCDQEPCLVDPDMIKAINQGNYGW
               *     **.  *  *        .*** ***   . *.* *  *** *..*. **.*.***
  A57480   111 QSWYPEGCIRDGLHYEEGSVIKENCNSCTCS-GQHWKCSQHVCLVLPELIEHINKGDYGW

DNA33223   158 QAGNHSAFWGMTLDEGIRYRLGTIRPSSSVMNMHEIYTVLNPGEVLPTAFEASEKWPNLI
                * *.* ******.** *.****. **  ...*.*.   *     **  * *  .   .
  A57480   170 TAQNYSQFWGMTLEEGFRFRLGTLPPSPVLLSMNEMRATLPETTDLPEFFIAFLQMAWMD

DNA33223   218 HEPLDQGNCAGSWAFSTAAVASDRVSIHSLGHMTPVLSPQNLLSCDTHQQQGCRGGRLDG
                  *** *******.**.**..* * *  *  ******.**    . **  * .*
  A57480   230 SWAIGSKNCAASWAFSTASVAADRIAIQSNGRYTANLSPQNLISCCAKNRHGCNSGSIDR

DNA33223   278 AWWFLRRRGVVSDHCYPFSGRERDEAGPAPPCMMHSRAMGRGKRQATAHCPNSYVNNNDI
               ***.**.**.**  ***     .*.      * * *.* ***** **  ***.   .* *
  A57480   290 AWWYLRKRGLVSHACYPLF---KDQNISNNTCAMTSKADGRGKRHATRPCPNNIEKSNRI

DNA33223   338 YQVTPVYRLGSNDKEIMKELMENGPVQALMEVHEDFFLYKGGIYSHTPVSLGRPERYRRH
               ** .* **. **. *****.*.******.*.****** ** *** *    .    *.**.
  A57480   347 YQCSPPYRVSSNETEIMKEIMQNGPVQAIMQVHEDFFHYKTGIYRHVISTNEESEKYRKL

DNA33223   398 GTHSVKITGWGEETLPDGRTLKYWTAANSWGPAWGERGHFRIVRGVNECDIESFVLGVWG
                **.**.****     *.  *.* ****** .*** *.***.***** ***  ..  **
  A57480   407 QTHAVKLTGWGTLKGARGQKEKFWIAANSWGKSWGENGYFRILRGVNESDIEKLIIAAWG

DNA33223   458 RVGMED
               ..   *
  A57480   467 QLTSSD
```

FIG. 21

DNA33473

```
>< /usr/seqdb2/sst/DNA/Dnaseqs.full1/ss.DNA33473 (1513 bases)>
< good sequence: 1-1513 (1513 bases)>
< insert: 147-1412 (1266 bases), 9 regions found>
<    5' pRK5 + lk: 82-901, 65 matches (100%), 65 consec>
<    3' pRK5 + lk: 1413-1566, 66 matches (100%), 66 consec>
<    pRK5D: 1-820, 102 matches (96%), 95 consec, 2 gaps>
<    pRK5D: 1415-1366, 84 matches (100%), 84 consec>
<    pRK5D: 92-920, 28 matches (100%), 28 consec>
<    pRK5B: 1-820, 134 matches (100%), 134 consec>
<    pRK5B: 1415-1357, 84 matches (100%), 84 consec>
<    3' cDNA linker: 1413-1566, 26 matches (100%), 26 consec>
<    5' cDNA linker: 131-950, 16 matches (100%), 16 consec>
GCTGGGGAC
><orf {trans=1-s, dir=f, res=1}>
ATGAGAGGCACACCGAAGACCCACCTCCTGGCCTTCTCCCTCCTCTGCCTCCTCTCAAAG
GTGCGTACCCAGCTGTGCCCGACACCATGTACCTGCCCCTGGCCACCTCCCCGATGCCCG
CTGGGAGTACCCCTGGTGCTGGATGGCTGTGGCTGCTGCCGGGTATGTGCACGGCGGCTG
GGGGAGCCCTGCGACCAACTCCACGTCTGCGACGCCAGCCAGGGCCTGGTCTGCCAGCCC
GGGGCAGGACCCGGTGGCCGGGGGGCCCTGTGCCTCTTGGCAGAGGACGACAGCAGCTGT
GAGGTGAACGGCCGCCTGTATCGGGAAGGGGAGACCTTCCAGCCCCACTGCAGCATCCGC
TGCCGCTGCGAGGACGGCGGCTTCACCTGCGTGCCGCTGTGCAGCGAGGATGTGCGGCTG
CCCAGCTGGGACTGCCCCCACCCCAGGAGGGTCGAGGTCCTGGGCAAGTGCTGCCCTGAG
TGGGTGTGCGGCCAAGGAGGGGGACTGGGGACCCAGCCCCTTCCAGCCCAAGGACCCCAG
TTTTCTGGCCTTGTCTCTTCCCTGCCCCCTGGTGTCCCCTGCCCAGAATGGAGCACGGCC
TGGGGACCCTGCTCGACCACCTGTGGGCTGGGCATGGCCACCCGGGTGTCCAACCAGAAC
CGCTTCTGCCGACTGGAGACCCAGCGCCGCCTGTGCCTGTCCAGGCCCTGCCCACCCTCC
AGGGGTCGCAGTCCACAAAACAGTGCCTTCTAGAGCCGGGCTGGGAATGGGGACACGGTG
TCCACCATCCCCAGCTGGTGGCCCTGTGCCTGGGCCCTGGGCTGATGGAAGATGGTCCGT
GCCCAGGCCCTTGGCTGCAGGCAACACTTTAGCTTGGGTCCACCATGCAGAACACCAATA
TTAACACGCTGCCTGGTCTGTCTGGATCCCGAGGTATGGCAGAGGTGCAAGACCTAGTCC
CCTTTCCTCTAACTCACTGCCTAGGAGGCTGGCCAAGGTGTCCAGGGTCCTCTAGCCCAC
TCCCTGCCTACACACACAGCCTATATCAAACATGCACACGGGCGAGCTTTCTCTCCGACT
TCCCCTGGGCAAGAGATGGGACAAGCAGTCCCTTAATATTGAGGCTGCAGCAGGTGCTGG
GCTGGACTGGCCATTTTTCTGGGGGTAGGATGAAGAGAAGGCACACAGAGATTCTGGATC
TCCTGCTGCCTTTTCTGGAGTTTGTAAAATTGTTCCTGAATACAAGCCTATGCGTGA
```

FIG. 22

SUBSTITUTE SHEET (RULE 26)

```
></usr/seqdb2/sst/DNA/Dnaseqs.min/ss.DNA33473
><subunit 1 of 1, 250 aa, 1 stop
><MW: 26825, pI: 8.36, NX(S/T): 0
MRGTPKTHLLAFSLLCLLSKVRTQLCPTPCTCPWPPPRCPLGVPLVLDGCGCCRVCARRL
GEPCDQLHVCDASQGLVCQPGAGPGGRGALCLLAEDDSSCEVNGRLYREGETFQPHCSIR
CRCEDGGFTCVPLCSEDVRLPSWDCPHPRRVEVLGKCCPEWVCGQGGGLGTQPLPAQGPQ
FSGLVSSLPPGVPCPEWSTAWGPCSTTCGLGMATRVSNQNRFCRLETQRRLCLSRPCPPS
RGRSPQNSAF
```

FIG. 23

[DNA30843]

CTGCAGGGGACATGAGAGGCACACCGAAGACCCACCTCCTGGCCTTCTC
CCTCCTCTGCCTCCTCTCAAAGGTGCGTACCCAGCTGTGCCCGACACCAT
GTACCTGCCCCTGGCCACCTCCCCGATGCCCGCTGGGAGTACCCCTGGTG
CTGGATGGCTGTGGCTGCTGCCGGGTATGTGCACGGCGGCTGGGGGAGCC
CTGCGACCAACTCCACGTCTGCGACGCCAGCCAGGGCCTGGTCTGCCAGC
CCGGGGCAGGACCCGGTGGCCGGGGGGCCCTGTGCCTCTTGGCAGAGGAC
GACAGCAGCTGTGAGGTGAACGGCCGCCTGTATCGGGAAGGGGAGACCTT
CCAGCCCCACTGCAGCATCCGCTGCCGCTGCGAGGACGGCGGCTTCACCT
GCGTGCCGCTGTGCAGCGAGGATGTGCGGCTGCCCAGCTGGGACTGCCCC
CACCCCAGGAGGGTCGAGGTCCTGGGCAAGTGCTGCCCTGAGTGGGTGTG
CGGCCAAGGAGGGGGACTGGGGACCAGCCCTTCCAGCCCAAGGACCCC
AGTTTTCTGGCCTTGTCTCTTCCCTGCCCCCTGGTGTCCCCTGCCCAGAA
TGGAGCACGGCCTGGGGACCCTGCTCGACCACCTGTGGGCTGGGCATGGC
CACCCGGGTGTCCAACCAGAACCGCTTCTGCCGACTGGAGACCCAGCGCC
GCCTGTGCCTGTCCAGGCCCTGCCCACCCTCCAGGGGTCGCAGTCCACAA
AACAGTGCCTTCTAGAGCCGGGCTGGGAATGGGGACACGGTGTCCACCAT
CCCCAGCTGGTGGCCCTGTGCCTGGGCCCTGGGCTGATGGAAGA

FIG. 24

SUBSTITUTE SHEET (RULE 26)

```
>4 CTGF_HUMAN Connective tissue growth factor precursor - homo sapien (349 aa)
 Score =  559 (196.8 bits), Expect = 2.3e-53, 2.3e-53
 Identities = 111/227 (48%), Positives = 137/227 (60%), at 49,16, Frame = +1

DNA33473 14 LLCLLSKVRT-QLCPTPCTCP WPPPRCPLGVPLVLDGCGCCRVCARRLGEPCDQLHVCD
            ** * *.    * *  ** **  * **** ** **********..*** * .   **
 CTGF_HUMAN   16 LLALCSRPAVGQNCSGPCRCPDEPAPRCPAGVSLVLDGCGCCRVCAKQLGELCTERDPCD

   DNA33473   72 ASQGLVCQPGAGPGGRGALCLLAEDDSSCEVNGRLYREGETFQPHCSIRCRCEDGGFTCV
                .** *  *.    .  .* *.* . *   * .** **.**  *  .* * **   *.
 CTGF_HUMAN   76 PHKGLFCDFGSPANRKIGVCT-AKDGAPCIFGGTVYRSGESFQSSCKYQCTCLDGAVGCM

   DNA33473  132 PLCSEDVRLPSWDCPHPRRVEVLGKCCPEWVCGQGGGLGTQPLPAQGPQFSG--LVSSLP
                 **** ****** *** ****.. **** **** .       .      **   .   *  .
CTGF_HUMAN  135 PLCSMDVRLPSPDCPFPRRVKLPGKCCEEWVCDE-----PKDQTVVGPALAAYRLEDTFG

  DNA33473  190 PG-----VPCPEWSTAWGPCSTTCGLGMATRVSNQNRFCRLETQRRLCLSRPC
                *         *   .* *  ** ***.*..***.* *  **** * ***. ***
CTGF_HUMAN  190 PDPTMIRANCLVQTTEWSACSKTCGMGISTRVTNDNASCRLEKQSRLCMVRPC
```

FIG. 25

GGAGCCGCCCTGGGTGTCAGCGGCTCGGCTCCCGCGCACGCTCCGGCCGTCGCGCAGCCT
CGGCACCTGCAGGTCCGTGCGTCCCGCGGCTGGCGCCCCTGACTCCGTCCCGGCCAGGGA
GGGCCATGATTTCCCTCCCGGGGCCCCTGGTGACCAACTTGCTGCGGTTTTTGTTCCTGG
GGCTGAGTGCCCTCGCGCCCCCCTCGCGGGCCCAGCTGCAACTGCACTTGCCCGCCAACC
GGTTGCAGGCGGTGGAGGGAGGGGAAGTGGTGCTTCCAGCGTGGTACACCTTGCACGGGG
AGGTGTCTTCATCCCAGCCATGGGAGGTGCCCTTTGTGATGTGGTTCTTCAAACAGAAAG
AAAAGGAGGATCAGGTGTTGTCCTACATCAATGGGGTCACAACAAGCAAACCTGGAGTAT
CCTTGGTCTACTCCATGCCCTCCCGGAACCTGTCCCTGCGGCTGGAGGGTCTCCAGGAGA
AAGACTCTGGCCCCTACAGCTGCTCCGTGAATGTGCAAGACAAACAAGGCAAATCTAGGG
GCCACAGCATCAAAACCTTAGAACTCAATGTACTGGTTCCTCCAGCTCCTCCATCCTGCC
GTCTCCAGGGTGTGCCCCATGTGGGGGCAAACGTGACCCTGAGCTGCCAGTCTCCAAGGA
GTAAGCCCGCTGTCCAATACCAGTGGGATCGGCAGCTTCCATCCTTCCAGACTTTCTTTG
CACCAGCATTAGATGTCATCCGTGGGTCTTTAAGCCTCACCAACCTTTCGTCTTCCATGG
CTGGAGTCTATGTCTGCAAGGCCCACAATGAGGTGGGCACTGCCCAATGTAATGTGACGC
TGGAAGTGAGCACAGGGCCTGGAGCTGCAGTGGTTGCTGGAGCTGTTGTGGGTACCCTGG
TTGGACTGGGGTTGCTGGCTGGGCTGGTCCTCTTGTACCACCGCCGGGGCAAGGCCCTGG
AGGAGCCAGCCAATGATATCAAGGAGGATGCCATTGCTCCCCGGACCCTGCCCTGGCCCA
AGAGCTCAGACACAATCTCCAAGAATGGGACCCTTTCCTCTGTCACCTCCGCACGAGCCC
TCCGGCCACCCCATGGCCCTCCCAGGCCTGGTGCATTGACCCCCACGCCCAGTCTCTCCA
GCCAGGCCCTGCCCTCACCAAGACTGCCCACGACAGATGGGGCCCACCCTCAACCAATAT
CCCCCATCCCTGGTGGGGTTTCTTCCTCTGGCTTGAGCCGCATGGGTGCTGTGCCTGTGA
TGGTGCCTGCCCAGAGTCAAGCTGGCTCTCTGGTATGATGACCCCACCACTCATTGGCTA
AAGGATTTGGGGTCTCTCCTTCCTATAAGGGTCACCTCTAGCACAGAGGCCTGAGTCATG
GGAAAGAGTCACACTCCTGACCCTTAGTACTCTGCCCCCACCTCTCTTTACTGTGGGAAA
ACCATCTCAGTAAGACCTAAGTGTCCAGGAGACAGAAGGAGAAGAGGAAGTGGATCTGGA
ATTGGGAGGAGCCTCCACCCACCCCTGACTCCTCCTTATGAAGCCAGCTGCTGAAATTAG
CTACTCACCAAGAGTGAGGGGCAGAGACTTCCAGTCACTGAGTCTCCCAGGCCCCCTTGA
TCTGTACCCCACCCCTATCTAACACCACCCTTGGCTCCCACTCCAGCTCCCTGTATTGAT
ATAACCTGTCAGGCTGGCTTGGTTAGGTTTTACTGGGGCAGAGGATAGGGAATCTCTTAT
TAAAACTAACATGAAATATGTGTTGTTTTCATTTGCAAATTTAAATAAAGATACATAATG
TTTGTATGAAAAA

FIG. 26

SUBSTITUTE SHEET (RULE 26)

MISLPGPLVTNLLRFLFLGLSALAPPSRAQLQLHLPANRLQAVEGGEVVLPAWYTLHGEV
SSSQPWEVPFVMWFFKQKEKEDQVLSYINGVTTSKPGVSLVYSMPSRNLSLRLEGLQEKD
SGPYSCSVNVQDKQGKSRGHSIKTLELNVLVPPAPPSCRLQGVPHVGANVTLSCQSPRSK
PAVQYQWDRQLPSFQTFFAPALDVIRGSLSLTNLSSSMAGVYVCKAHNEVGTAQCNVTLE
VSTGPGAAVVAGAVVGTLVGLGLLAGLVLLYHRRGKALEEPANDIKEDAIAPRTLPWPKS
SDTISKNGTLSSVTSARALRPPHGPPRPGALTPTPSLSSQALPSPRLPTTDGAHPQPISP
IPGGVSSSGLSRMGAVPVMVPAQSQAGSLV

FIG. 27

><DNA30955: 4 members (4 incyte) 387 bp, 0 gaps, 144 bp orf (+3)>
<orf {trans=1-s, dir=f, res=1}>

GTCTACTCCATGCCCTCCCGGAACCTGTCCCTGCGGCTGGAGGGTCTCCA
GGAGAAAGACTCTGGCCCCTACAGCTGCTCCGTGAATGTGCAAGACAAAC
AAGGCAAATCTAGGGGCCACAGCATCAAAACCTTAGAACTCAATGTACTG
GTTCCTCCAGCTCCTCCATCCTGCCGTCTCCAGGGTGTGCCCCATGTGGG
GGCAAACGTGACCCTGAGCTGCCAGTCTCCAAGGAGTAAGCCCGCTGTCC
AATACCAGTGGGATCGGCAGCTTCCATCCTTCCAGACTTTCTTTGCACCA
GCATTAGATGTCATCCGTGGGTCTTTAAGCCTCACCAACCTTTCGTCTTC
CATGGCTGGAGTCTATGTCTGCAAGGCCCACAATGAG

FIG. 28

SUBSTITUTE SHEET (RULE 26)

```
2 HSU90716 - 1 cell surface protein HCAR - Homo sapiens (365 aa)
 Score = 311 (109.5 bits), Expect = 2.3e-26, P = 2.3e-26
 Identities = 105/378 (27%), Positives = 180/378 (47%), at 162,3, Frame = +3

  DNA35639    13 LRFLFLGLSALAPPSRAQLQLHLPANRLQAVEGGEVVLPAWYTLHGEVSSSQPWEVPFVM
                 *   *. *   .   .*. * .  *   ..  .*   **  .**  *     * .. ...
HSU90716-1     3 LLLCFVLLCGVVDFARS-LSITTPEEMIEKAKGETAYLPCKFTLSPE--DQGPLDIEWLI

  DNA35639    73 WFFKQKEKEDQVLSYING-------VTTSKPGVSLVYS-MPSRNLSLRLEGLQEKDSGPY
                    .* ***.   .*            *  *    . . * . *. .  **  * * *
HSU90716-1     60 -SPADNQKVDQVIILYSGDKIYDDYYPDLKGRVHFTSNDLKSGDASINVTNLQLSDIGTY

  DNA35639   125 SCSVNVQDKQGKSRGHSIKTLELNVLVPPAPPSCRLQGVPHVGANVTLSCQSPRSKPAVQ
                 * *      *. * . * . * *** *.   * . *   .*.. . *.      .*
HSU90716-1  119 QCKVK------KAPGVANKKIHLVVLVYPSGARCYVDGSEEIGSDFKIKCEPKEGSLPLQ

  DNA35639   185 YQWDRQLPSFQTFFAPALDVIRGSLSLTNLSSSMAGVYVCKAHNEVGTAQCNVTLEVSTG
                 *.*  .   *  .   .   ..    .*. * **  .* * *   * **. **  . * *
HSU90716-1  173 YEWQKLSDSQKMPTSWLAEMTSSVISVKNASSEYSGTYSCTVRNRVGSDQCLLRLNVVPP

  DNA35639   245 PG-AAVVAGAVVGTLVGLGLLAGLVLLYHRRGKALEEPANDIKEDAIAPRTLPWPKSSDT
                   * ..***..***. * *. **..   *. .  *.   ..  *      .* ***  .
HSU90716-1  233 SNKAGLIAGAIIGTLLALALI-GLIIFCCRKKRREEKYEKEVHHDI--REDVPPPKSRTS

  DNA35639   304 ISKNGTLSSVTSARALRPPHGPPRPGALTPTPSLSSQALPSPRLPTTDGAHPQ-PISPIP
                 ...   *. .*  .. * .       .                .**    *    ** * * *
HSU90716-1  290 TARSYIGSNHSSLGSMSPSN-------MEGYSKTQYNQVPSEDFERT----PQSPTLP-P

  DNA35639   363 GGVSSSGLSRMGAVPVMVPAQSQAGSLV
                   *..  ******.***.****. **.*
HSU90716-1  338 AKVAAPNLSRMGAIPVMIPAQSKDGSIV
```

FIG. 29

```
   1 TGAGACCCTC CTGCAGCCTT CTCAAGGGAC AGCCCCACTC TGCCTCTTGC TCCTCCAGGG CAGCACCATG CAGCCCCTGT GGCTCTGCTG GGCACTCTGG
     ACTCTGGGAG GACGTCGGAA GAGTTCCCTG TCGGGGTGAG ACGGAGAACG AGGAGGTCCC GTCGTGGTAC GTCGGGGACA CCGAGACGAC CCGTGAGACC
   1                                                                                 M   Q  P  L  W   L  C  W   A  L  W
                                                                                     MET (trans=1-s, dir=f, res=1)

 101 GTGTTGCCCC TGGCCAGCCC CGGGGCCGCC CTGACCGGGG AGCAGCTCCT GGGCAGCCTG CTGCGGCAGC TGCAGCTCAA AGAGGTGCCC ACCCTGGACA
     CACAACGGGG ACCGGTCGGG GCCCCGGCGG GACTGGCCCC TCGTCGAGGA CCCGTCGGAC GACGCCGTCG ACGTCGAGTT TCTCCACGGG TGGGACCTGT
  12 V  L  P  L   A  S  P   G  A  A   L  T  G  E   Q  L  L   G  S  L   L  R  Q  L   Q  L  K   E  V  P   T  L  D  R

 201 GGGCCGACAT GGAGGAGCTG GTCATCCCCA CCCACGTGAG GGCCCAGTAC GTGGCCCTGC TGCAGCGCAG CCACGGGGAC CGCTCCCGCG GAAAGAGGTT
     CCCGGCTGTA CCTCCTCGAC CAGTAGGGGT GGGTGCACTC CCGGGTCATG CACCGGGACG ACGTCGCGTC GGTGCCCCTG GCGAGGGCGC CTTTCTCCAA
  46   A  D  M   E  E  L   V  I  P   T   H  V  R   A  Q  Y   V  A  L  L   Q  R  S   H  G  D   R  S  R  G   K  R  F

 301 CAGCCAGAGC TTCCGAGAGG TGGCCGGCAG GTTCCTGGCG TTGGAGGCCA GCACACACCT GCTGGTGTTC GGCATGGAGC AGCGGCTGCC GCCCAACAGC
     GTCGGTCTCG AAGGCTCTCC ACCGGCCGTC CAAGGACCGC AACCTCCGGT CGTGTGTGGA CGACCACAAG CCGTACCTCG TCGCCGACGG CGGGTTGTCG
  79 S  Q  S   F  R  E  V   A  G  R   F  L  A   L  E  A  S   T  H  L   L  V  F   G  M  E  Q   R  L  P   P  N  S

 401 GAGCTGGTGC AGGCCGTGCT GCGGCTCTTC CAGGAGCCGG TCCCCAAGGC CGCGCTGCAC AGGCACGGGC GGCTGTCCCC GCGCAGCGCC CGGGCCCGGG
     CTCGACCACG TCCGGCACGA CGCCGAGAAG GTCCTCGGCC AGGGGTTCCG GCGCGACGTG TCCGTGCCCG CCGACAGGGG CGCGTCGCGG GCCCGGGCCC
 112 E  L  V  Q   A  V  L   R  L  F   Q  E  P  V   P  K  A   A  L  H   R  H  G  R   L  S  P   R  S  A   R  A  R  V

 501 TGACCGTCGA GTGGCTGCGC GTCCGCGACG ACGGCTCCAA CCGCACCTCC CTCATCGACT CCAGGCTGGT GTCCGTCCAC GAGAGCGGCT GGAAGGCCTT
     ACTGGCAGCT CACCGACGCG CAGGCGCTGC TGCCGAGGTT GGCGTGGAGG GAGTAGCTGA GGTCCGACCA CAGGCAGGTG CTCTCGCCGA CCTTCCGGAA
 146   T  V  E   W  L  R   V  R  D  D   G  S  N   R  T  S   L  I  D  S   R  L  V   S  V  H   E  S  G  W   K  A  F
```

FIG. 30A

```
601 CGACGTGACC GAGGCCGTGA ACTTCTGGCA GCAGCTGAGC CGGCCCCGGC AGCCGCTGCT GCTACAGGTG TCGGTGCAGA GGGAGCATCT GGGCCCGCTG
    GCTGCACTGG CTCCGGCACT TGAAGACCGT CGTCGACTCG GCCGGGGCCG TCGGCGACGA CGATGTCCAC AGCCACGTCT CCCTCGTAGA CCCGGGCGAC
179  D  V  T   E  A  V  N   F  W  Q   Q  L  S   R  P  R  Q   P  L  L   L  Q  V   S  V  Q  R   E  H  L   G  P  L

701 GCGTCCGGCG CCCACAAGCT GGTCCGCTTT GCCTCGCAGG GGGCGCCAGC CGGGCTTGGG GAGCCCCAGC TGGAGCTGCA CACCCTGGAC CTTGGGGACT
    CGCAGGCCGC GGGTGTTCGA CCAGGCGAAA CGGAGCGTCC CCCGCGGTCG GCCCGAACCC CTCGGGGTCG ACCTCGACGT GTGGGACCTG GAACCCCTGA
212 A  S  G  A   H  K  L   V  R  F   A  S  Q  G   A  P  A   G  L  G   E  P  Q  L   E  L  H   T  L  D   L  G  D  Y

801 ATGGAGCTCA GGGCGACTGT GACCCTGAAG CACCAATGAC CGAGGGCACC CGCTGCTGCC GCCAGGAGAT GTACATTGAC CTGCAGGGGA TGAAGTGGGC
    TACCTCGAGT CCCGCTGACA CTGGGACTTC GTGGTTACTG GCTCCCGTGG GCGACGACGG CGGTCCTCTA CATGTAACTG GACGTCCCCT ACTTCACCCG
246   G  A  Q   G  D  C   D  P  E  A   P  M  T   E  G  T   R  C  C  R   Q  E  M   Y  I  D   L  Q  G  M   K  W  A

901 CGAGAACTGG GTGCTGGAGC CCCCGGGCTT CCTGGCTTAT GAGTGTGTGG GCACCTGCCG GCAGCCCCCG GAGGCCCTGG CCTTCAAGTG GCCGTTTCTG
    GCTCTTGACC CACGACCTCG GGGGCCCGAA GGACCGAATA CTCACACACC CGTGGACGGC CGTCGGGGGC CTCCGGGACC GGAAGTTCAC CGGCAAAGAC
279  E  N  W   V  L  E  P   P  G  F   L  A  Y   E  C  V  G   T  C  R   Q  P  P   E  A  L  A   F  K  W   P  F  L

1001 GGGCCTCGAC AGTGCATCGC CTCGGAGACT GACTCGCTGC CCATGATCGT CAGCATCAAG GAGGGAGGCA GGACCAGGCC CCAGGTGGTC AGCCTGCCCA
     CCCGGAGCTG TCACGTAGCG GAGCCTCTGA CTGAGCGACG GGTACTAGCA GTCGTAGTTC CTCCCTCCGT CCTGGTCCGG GGTCCACCAG TCGGACGGGT
312  G  P  R  Q   C  I  A   S  E  T   D  S  L  P   M  I  V   S  I  K   E  G  G  R   T  R  P   Q  V  V   S  L  P  N

1101 ACATGAGGGT GCAGAAGTGC AGCTGTGCCT CGGATGGTGC GCTCGTGCCA AGGAGGCTCC AGCCATAGGC GCCTAGTGTA GCCATCGAGG GACTTGACTT
     TGTACTCCCA CGTCTTCACG TCGACACGGA GCCTACCACG CGAGCACGGT TCCTCCGAGG TCGGTATCCG CGGATCACAT CGGTAGCTCC CTGAACTGAA
346   M  R  V   Q  K  C   S  C  A  S   D  G  A   L  V  P   R  R  L  Q   P  O

1201 GTGTGTGTTT CTGAAGTGTT CGAGGGTACC AGGAGAGCTG GCGATGACTG AACTGCTGAT GGACAAATGC TCTGTGCTCT CTAGTGAGCC CTGAATTTGC
     CACACACAAA GACTTCACAA GCTCCCATGG TCCTCTCGAC CGCTACTGAC TTGACGACTA CCTGTTTACG AGACACGAGA GATCACTCGG GACTTAAACG

1301 TTCCTCTGAC AAGTTACCTC ACCTAATTTT TGCTTCTCAG GAATGAGAAT CTTTGGCCAC TGGAGAGCCC TTGCTCAGTT TTCTCTATTC TTATTATTCA
     AAGGAGACTG TTCAATGGAG TGGATTAAAA ACGAAGAGTC CTTACTCTTA GAAACCGGTG ACCTCTCGGG AACGAGTCAA AAGAGATAAG AATAATAAGT

1401 CTGCACTATA TTCTAAGCAC TTACATGTGG AGATACTGTA ACCTGAGGGC AGAAAGCCCA NTGTGTCATT GTTTACTTGT CCTGTCACTG GATCTGGGCT
     GACGTGATAT AAGATTCGTG AATGTACACC TCTATGACAT TGGACTCCCG TCTTTCGGGT NACACAGTAA CAAATGAACA GGACAGTGAC CTAGACCCGA

1501 AAAGTCCTCC ACCACCACTC TGGACCTAAG ACCTGGGGTT AAGTGTGGGT TGTGCATCCC CAATCCAGAT AATAAAGACT TTGTAAAACA TGAATAAAAC
     TTTCAGGAGG TGGTGGTGAG ACCTGGATTC TGGACCCCAA TTCACACCCA ACACGTAGGG GTTAGGTCTA TTATTTCTGA AACATTTTGT ACTTATTTTG

1601 ACATTTTATT CTAAAA
     TGTAAAATAA GATTTT
```

FIG. 30B

DNA28722: 27 members (23 incyte, 4 est) 1021 bases
possible alternative splice with R09637

CAACGACCTACATGGCGAGGGACCCCCGACACCAGTGCCTCCCGCTCAA
CACCCCAACCCCTGCCCACCAGGCTATGAGCCCGACGATCAGGACAGCTG
TGTGGATGTGGACGAGTGTGCCCAGGCCCTGCACGACTGTCGCCCCAGCC
AGGACTGCCATAACTTGCCTGGCTCCTATCAGTGCACCTGCCCTGATGGT
TACCGCAAGATCGGGCCCGAGTGTGTGGACATAGACGAGTGCCGCTACCG
CTACTGCCAGCACCGCTGCGTGAACCTGCCTGGCTCCTTCCGCTGCCAGT
GCGAGCCGGGCTTCCAGCTGGGGCCTAACAACCGCTCCTGTGTTGATGTG
AACGAGTGTGACATGGGGGCCCCATGCGAGCAGCGCTGCTTCAACTCCTA
TGGGACCTTCCTGTGTCGCTGCCACCAGGGCTATGAGCTGCATCGGGATG
GCTTCTCCTGCAGTGATATTGATGAGTGTAGCTACTCCAGCTACCTCTGT
CAGTACCGCTGCGTCAACGAGCCAGGGCCGTTTCTCCTGGCCACTGCC
CACAGGGTTACCAGCTGCTGGCCACACGCCTCTGCCAAGACATTG
ATGAGTGTGAGTCTGGTGCGCACCAGTGCTCCGAGGCCCAAACC
TGTGTCAACTTCCATGGGGGCTACCGCTGCGTGGACACCAACCGCTGCG
TGGAGCCCTACATCCAGGTCTCTGAGAACCGCTGTCTCTGCCCGGCCTCC
AACCCTCTATGTCGAGAGCAGCCTTCATCCATTGTGCACCGCTACAT
GACCATCACCTCGGAGCGGAGAGACCCGCTCGACCGTGTTCCAGATCCA
GGCGAACCTCCGTCTACCCCGGTGCCTACAATGCCTTTCAGAT
CCGTGCTGGAAACTCGCAGGGGGACTTTTACATTAGGCAAATCA
ACAACGTCAGCGCCATGCTGGTCCTCGCCCGGCCGGTGGACGGGNCCCCG
GGAGTACGTTGCTGGACCTGGAGAATGGTCACATGAATTCCTCATGAGCT
ACG

FIG. 31

```
/usr/seqdb2/sst/DNA/Dnaseqs.min/ss.DNA33461 (1616 bp)
Scoring parameters: T=12, S=66, S2=36, Matrix: BLOSUM62
Database: /usr/seqdb/blast/dblast (356,412 entries, 87,954,021 aa)

Sequences producing High-scoring Segment Pairs:                Frame Score Match Pct
  1 EBAF_HUMAN  Endometrial bleeding-associated factor prec..  +2   1761   337  92
  2 MMAJ82_1    Lefty protein - Mus musculus                   +2   1594   299  82
  3 MMAJ83_1    Lefty protein - Mus musculus                   +2   1594   299  82
  4 LEFT_MOUSE  Lefty protein precursor - mus musculus         +2   1594   299  82
  5 GEN14630    TGF beta-family protein lefty - mouse(murine)  +2   1587   298  82

>1 EBAF_HUMAN  Endometrial bleeding-associated factor precursor - homo (370 aa)
 Score = 1761 (619.9 bits), Expect = 1.1e-180, P = 1.1e-180
 Identities = 337/365 (92%), Positives = 346/365 (94%), at 68,1, Frame = +2

  DNA33461     68 MQPLWLCWALWVLPLASPGAALTGEQLLGSLLRQLQLKEVPTLDRADMEELVIPTHVRAQ
                  * ************** ****** **** ******** *** *******.**** *****
EBAF_HUMAN      1 MWPLWLCWALWVLPLAGPGAALTEEQLLASLLRQLQLSEVPVLDRADMEKLVIPAHVRAQ

  DNA33461    248 YVALLQRSHGDRSRGKRFSQSFREVAGRFLALEASTHLLVFGMEQRLPPNSELVQAVLRL
                  ** **.*  ************************ ****************************
EBAF_HUMAN     61 YVVLLRRD-GDRSRGKRFSQSFREVAGRFLASEASTHLLVFGMEQRLPPNSELVQAVLRL

  DNA33461    428 FQEPVPKAALHRHGRLSPRSARARVTVEWLRVRDDGSNRTSLIDSRLVSVHESGWKAFDV
                  ******. ********** . .******** ****************************
EBAF_HUMAN    120 FQEPVPQGALHRHGRLSPAAPKARVTVEWL-VRDDGSNRTSLIDSRLVSVHESGWKAFDV

  DNA33461    608 TEAVNFWQQLSRPRQPLLLQVSVQREHLGPLASGAHKLVRFASQGAPAGLGEPQLELHTL
                  ************** .***.**************************************
EBAF_HUMAN    179 TEAVNFWQQLSRPPEPLLVQVSVQREHLGPLASGAHKLVRFASQGAPAGLGEPQLELHTL
```

FIG. 32A

SUBSTITUTE SHEET (RULE 26)

```
DNA33461     788 DLGDYGAQGDCDPEAPMTEGTRCCRQEMYIDLQGMKWAENWVLEPPGFLAYECVGTCRQP
                 ** ********************************.******************.**
EBAF_HUMAN   239 DLRDYGAQGDCDPEAPMTEGTRCCRQEMYIDLQGMKWAKNWVLEPPGFLAYECVGTCQQP

DNA33461     968 PEALAFKWPFLGPRQCIASETDSLPMIVSIKEGGRTRPQVVSLPNMRVQKCSCASDGALV
                 ****** ************** ************************************
EBAF_HUMAN   299 PEALAFNWPFLGPRQCIASETASLPMIVSIKEGGRTRPQVVSLPNMRVQKCSCASDGALV

DNA33461    1148 PRRLQ
                 *****
EBAF_HUMAN   359 PRRLQ
```

FIG. 32B

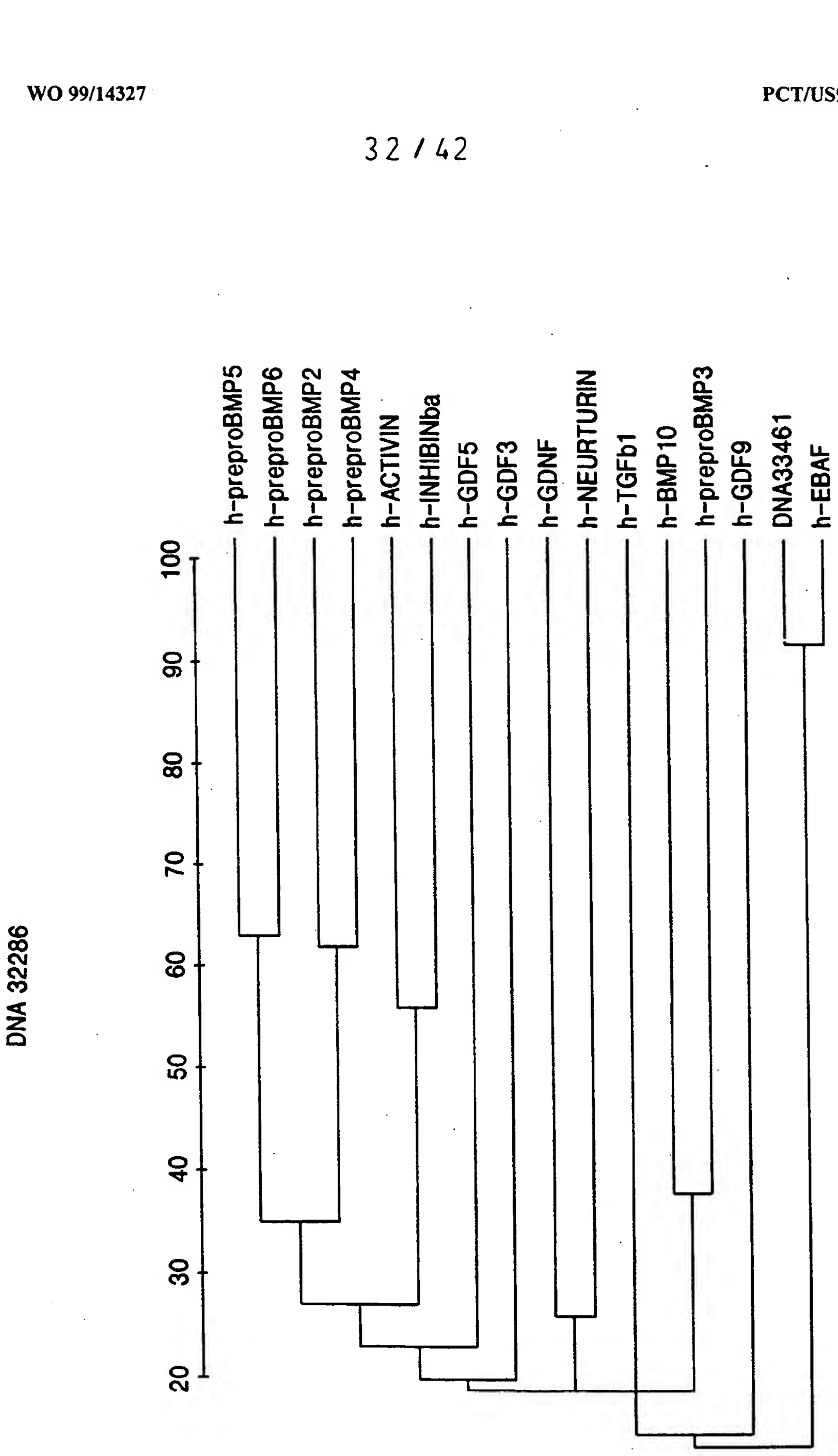
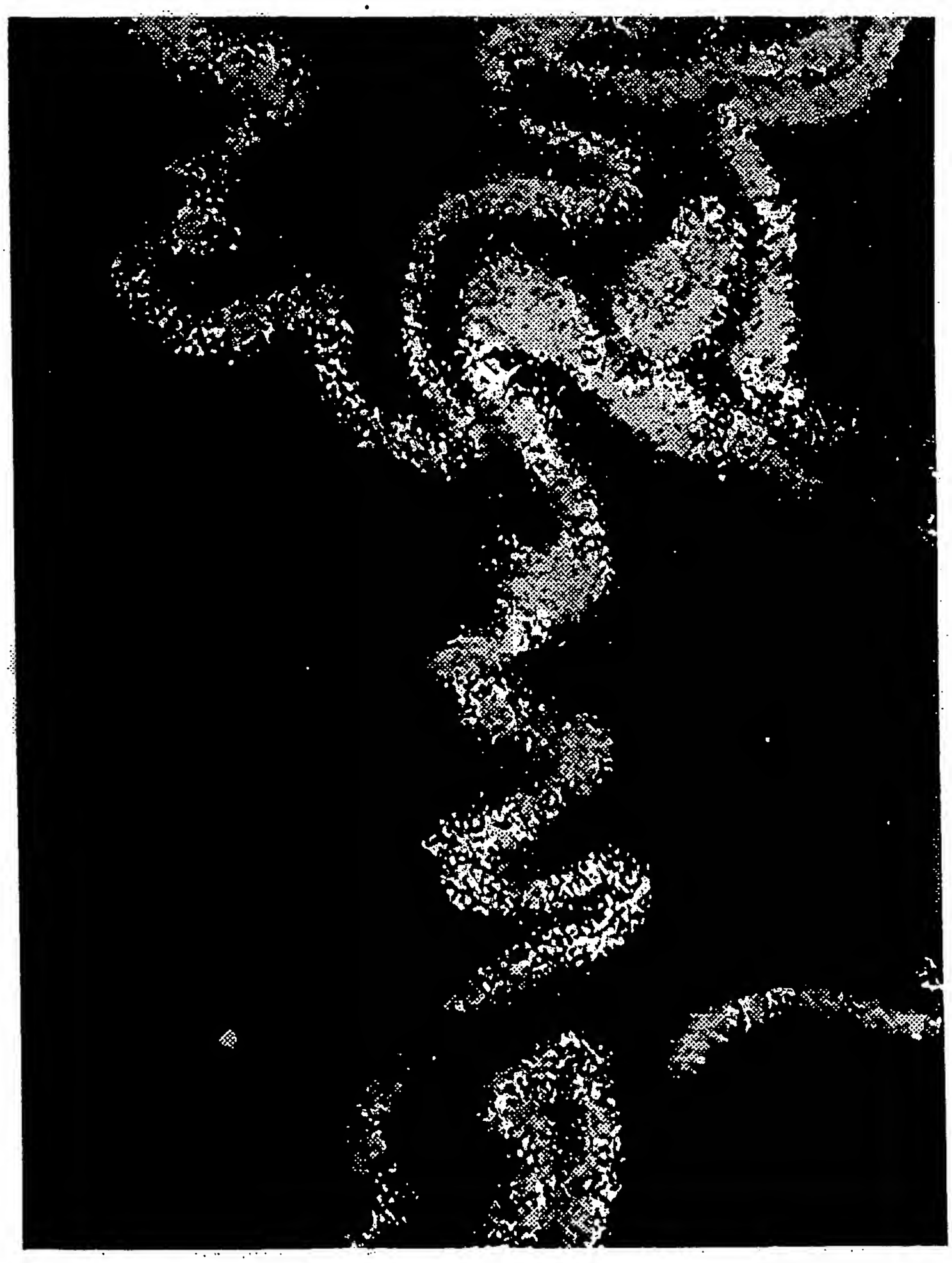

DNA 32286
20
30
40
50
60
70
80
90
100
h-preproBMP5
h-preproBMP6
h-preproBMP2
h-preproBMP4
h-ACTIVIN
h-INHIBINba
h-GDF5
h-GDF3
h-GDNF
h-NEURTURIN
h-TGFb1
h-BMP10
h-preproBMP3
h-GDF9
DNA33461
h-EBAF

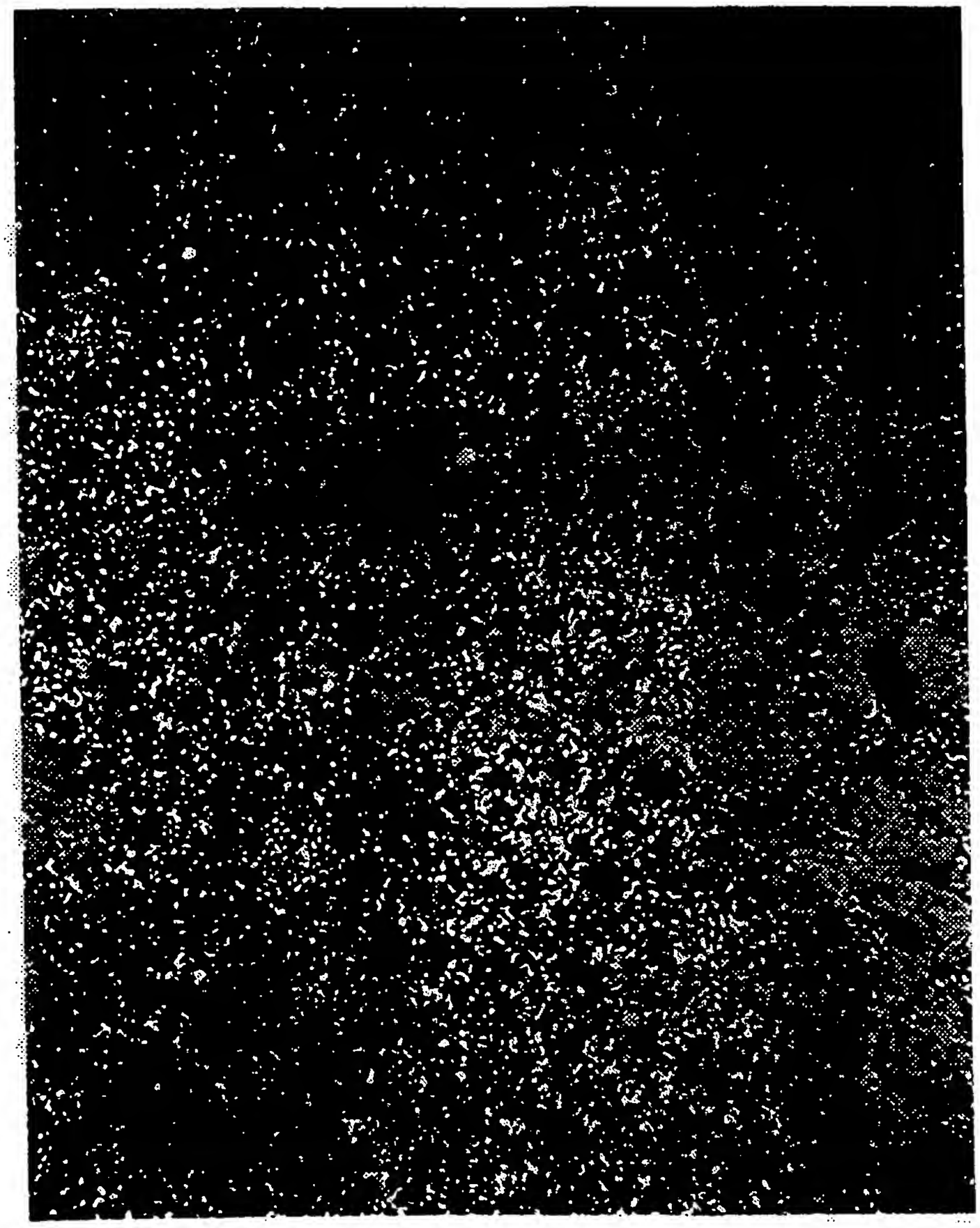
FIG. 33



DNA 49435

In adult tissues there is an extremely high level of expression over the gallbladder epithelium.

FIG. 34



DNA 32286

Moderate expression in placental stromal cells in membranous tissues.

FIG. 35

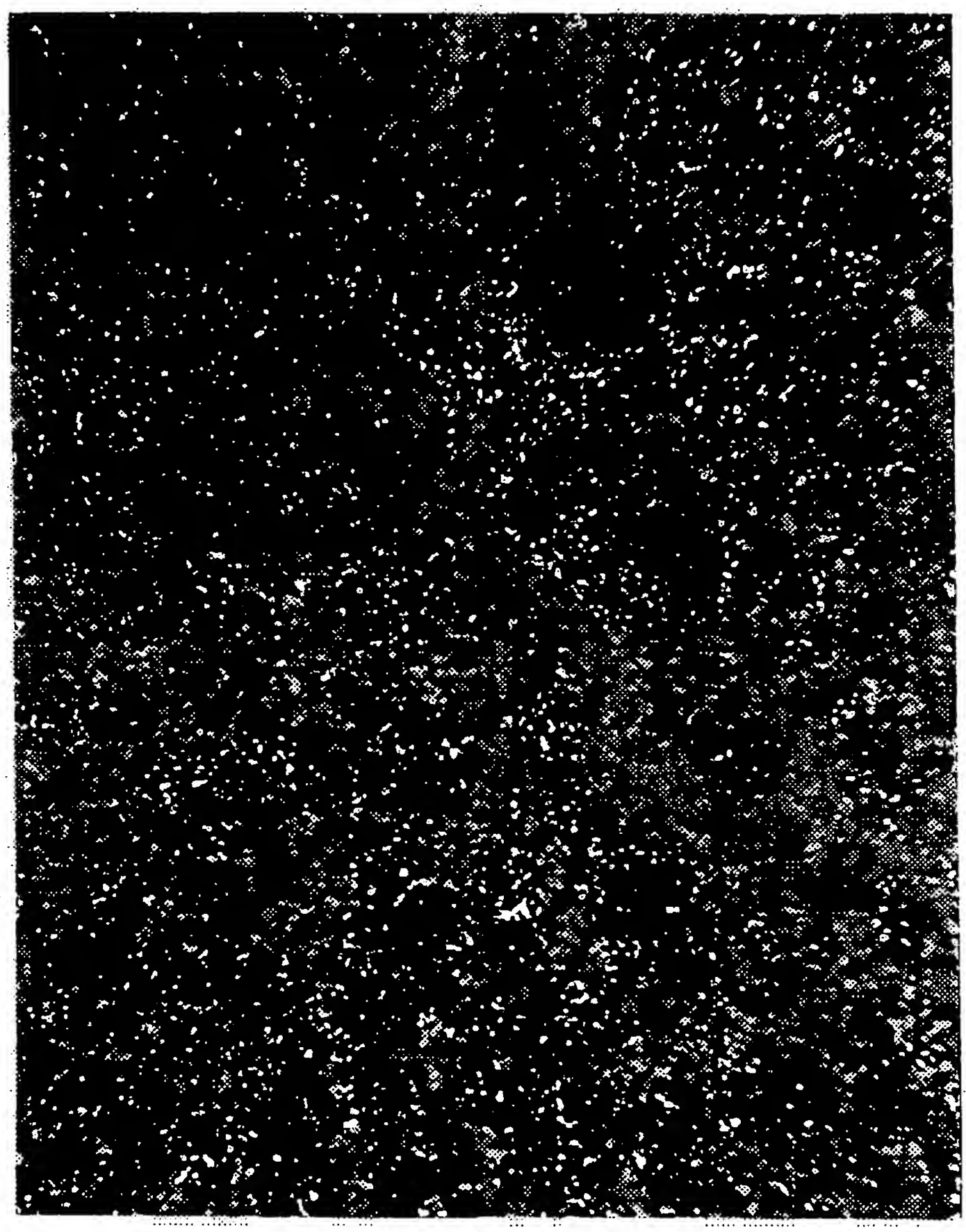
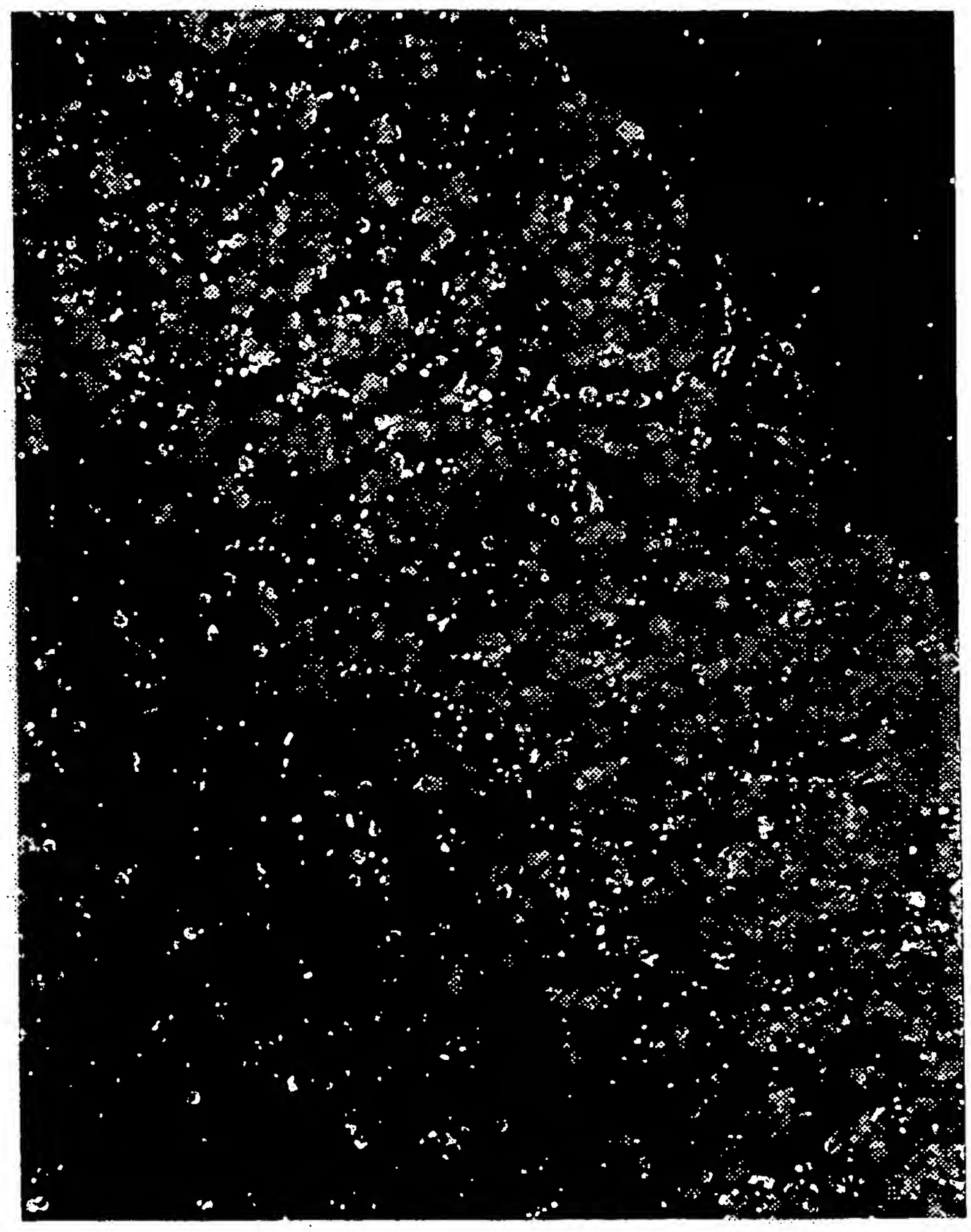
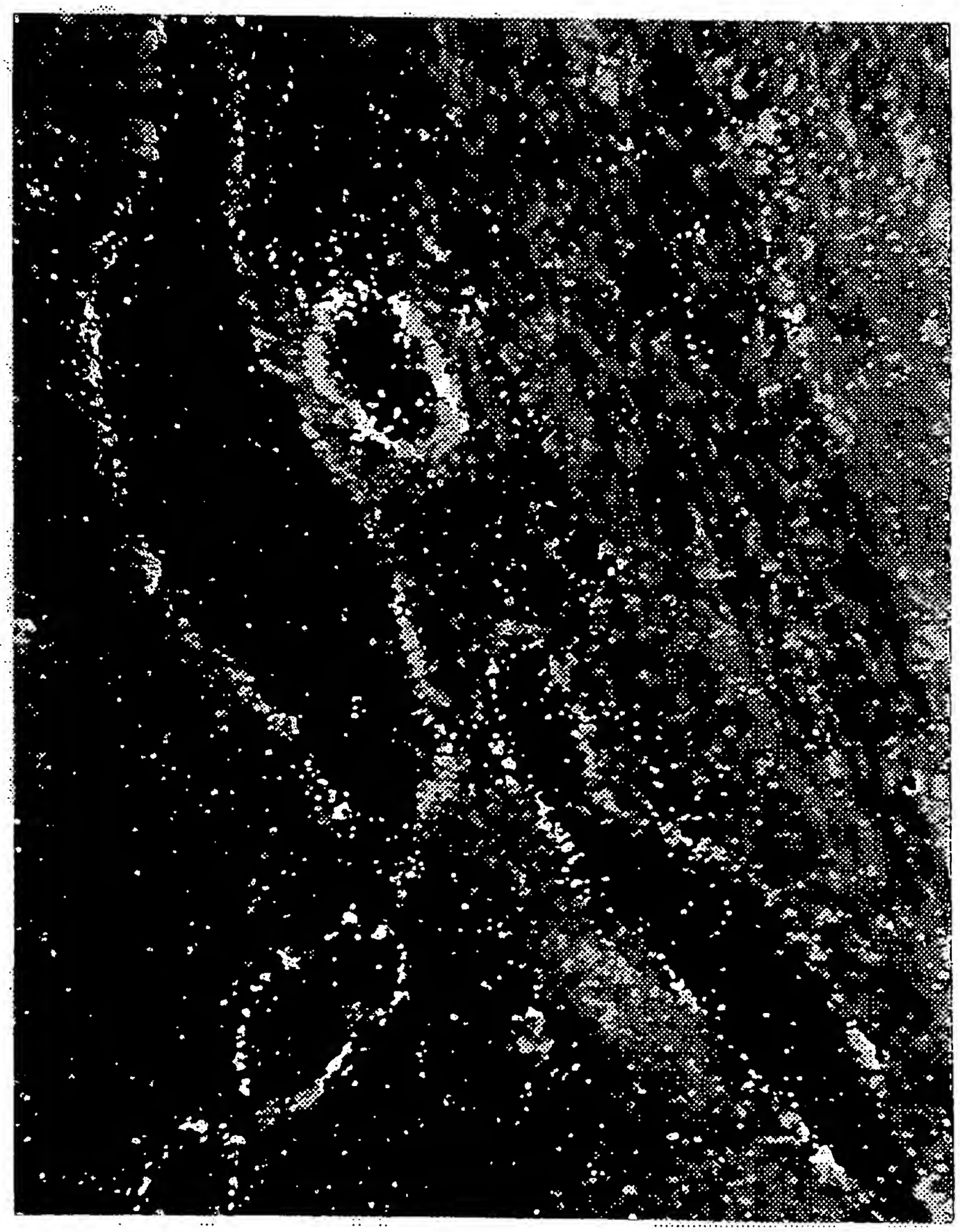


FIG. 36



Low level expression in cortical neurones.

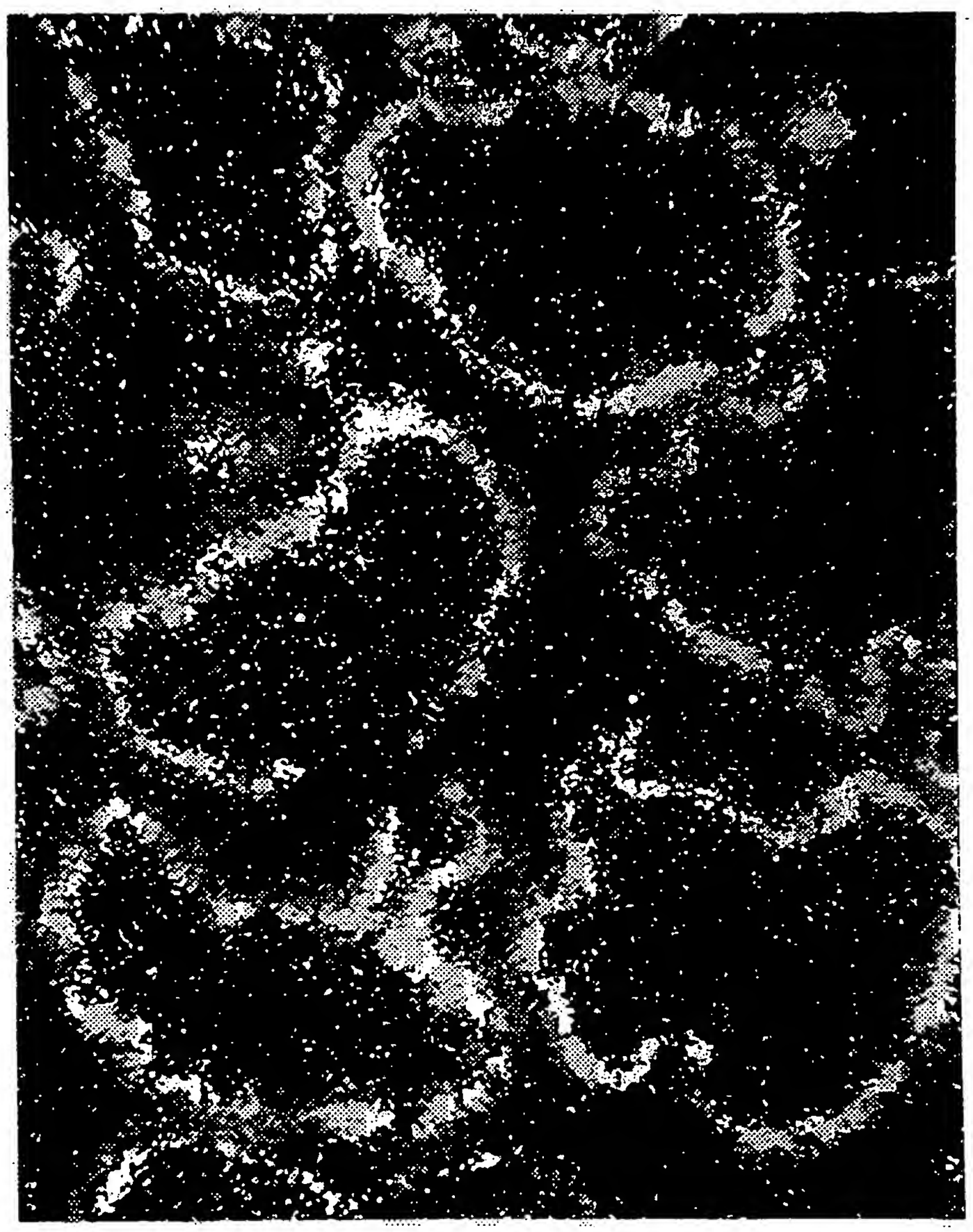
FIG. 37



DNA 33223

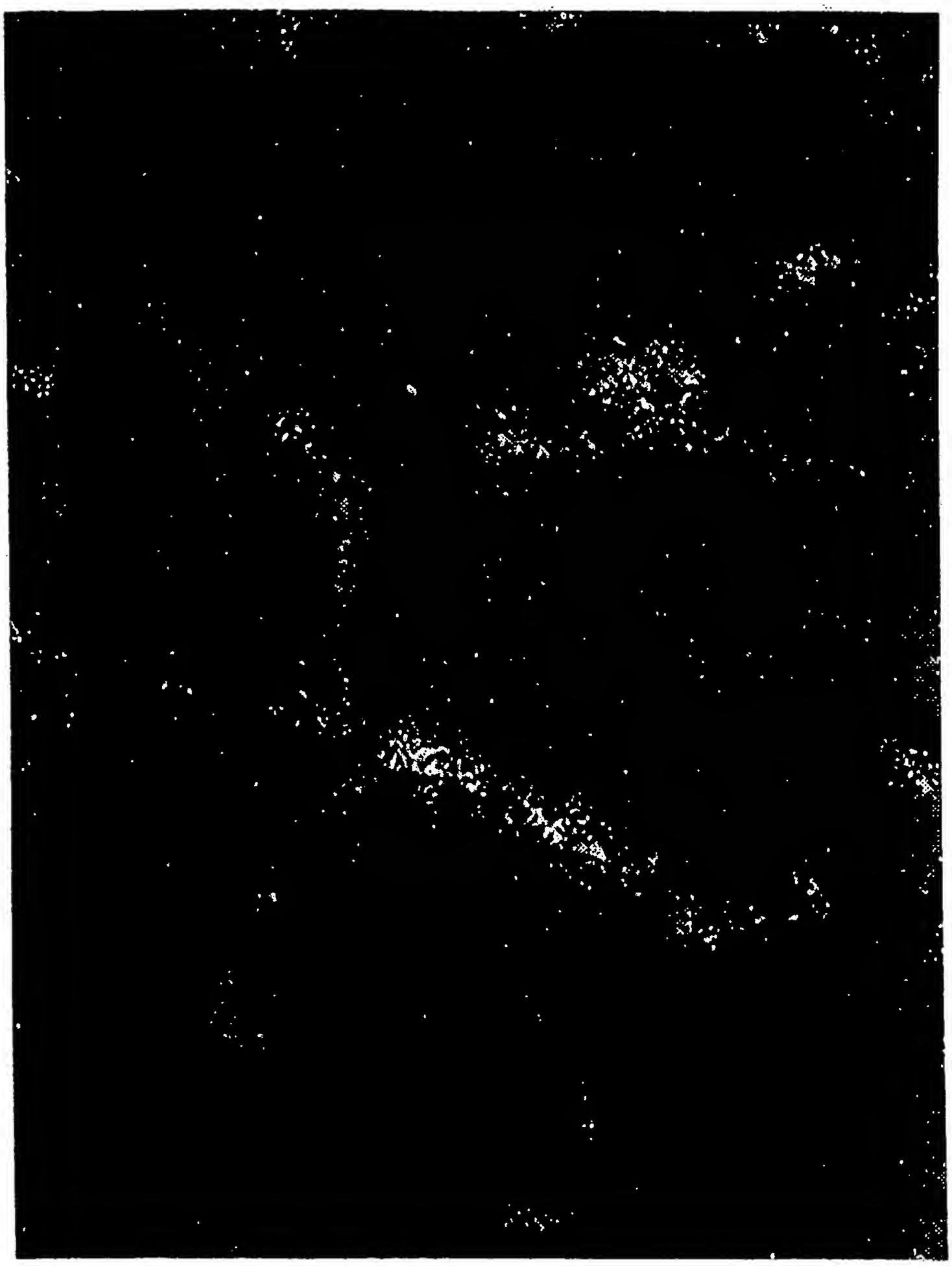
Sections show an intense signal associated with arterial and venous vessels in the fetus. In arteries the signal appeared to be confined to smooth-muscle/pericytic cells.

FIG. 38



Strong expression was also seen in cells within placental trophoblastic villi, these cells lie between the trophoblast and the fibroblast-like cells that express HGF-uncertain histogenesis.

FIG. 39



DNA 35639

Strongly expressed in fetal vascular endothelium.

FIG. 40

CACCTCAAGTGTGTAGACATTGATGAGTGTGGCACAGAGGGAGCCAACTG
TGGAGCTGACCAATTCTGCGTGAACACTGAGGGCTCCTATGAGTGCCGAG
ACTGTGCCAAGGCCTGCCTAGGCTGCATGGGGGCAGGGCCAGGTCGCTGT
AAGAAGTGTAGCCCTGGCTATCAGCAGGTGGGCTCCAAGTGTCTCGATGT
GGATGAGTGTGAGACAGAGGTGTGTCCGGGAGAGAACAAGCAGTGTGAAA
ACACCGAGGGCGGTTATCGCTGCATCTGTGCCGAGGGCTACAAGCAGATG
GAA GGCATCT GTGTGAAGGA GCAGATCCC AGAGTCAGCAGGCTTCT
TCTCAGAGATTGACAGAAGACGAAGTTTGGTGGTTGCTTGCAGCAAGATG
TTCCTTTTGGCATCATCATCTGTTGCACTGGCCACGCTGGCTTGCTAAGG
GGC

FIG. 41

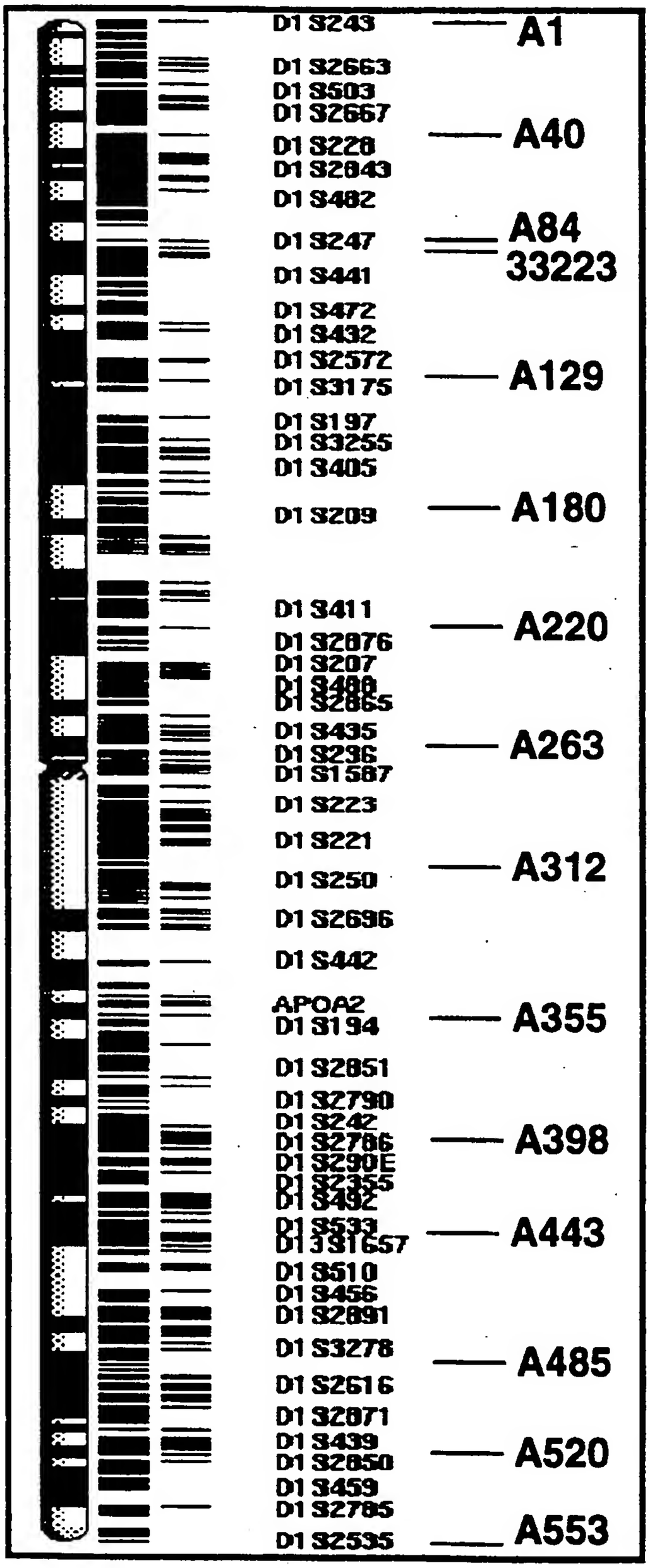

Chromosome 1
D1 S243
A1
D1 S2663
D1 S503
D1 S2667
A40
D1 S228
D1 S2843
D1 S482
A84
D1 S247
33223
D1 S441
D1 S472
D1 S432
D1 S2572
A129
D1 S3175
D1 S197
D1 S3255
D1 S405
A180
D1 S209
D1 S411
A220
D1 S2876
D1 S207
D1 S486
D1 S2865
D1 S435
A263
D1 S236
D1 S1587
D1 S223
D1 S221
A312
D1 S250
D1 S2696
D1 S442
APOA2
A355
D1 S194
D1 S2851
D1 S2790
D1 S242
A398
D1 S2786
D1 S290E
D1 S2355
D1 S492
D1 S533
A443
D1 S1657
D1 S510
D1 S456
D1 S2891
D1 S3278
A485
D1 S2616
D1 S2871
D1 S439
A520
D1 S2850
D1 S459
D1 S2785
A553
D1 S2535

FIG. 42

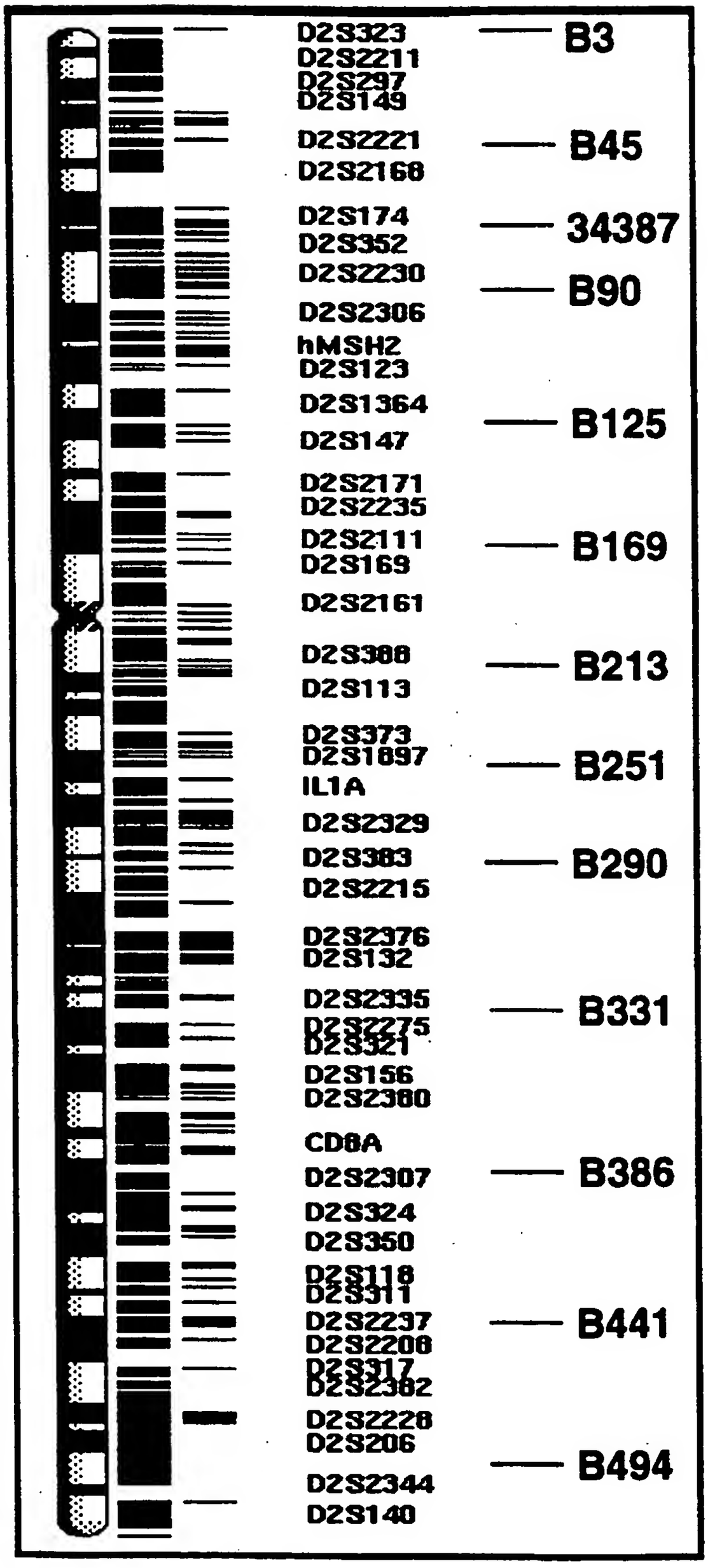

Chromosome 2
D2S323
D2S2211
D2S297
D2S149
D2S2221
D2S2168
D2S174
D2S352
D2S2230
D2S2306
hMSH2
D2S123
D2S1364
D2S147
D2S2171
D2S2235
D2S2111
D2S169
D2S2161
D2S388
D2S113
D2S373
D2S1897
IL1A
D2S2329
D2S383
D2S2215
D2S2376
D2S132
D2S2335
D2S2275
D2S321
D2S156
D2S2380
CD8A
D2S2307
D2S324
D2S350
D2S118
D2S311
D2S2237
D2S2208
D2S317
D2S2382
D2S2228
D2S206
D2S2344
D2S140
B3
B45
34387
B90
B125
B169
B213
B251
B290
B331
B386
B441
B494

FIG. 43

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60/062,816	24 October 1997 (24.10.97)	US
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(71) Applicant *(for all designated States except US)*: GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080–4990 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BOTSTEIN, David [US/US]; 2539 Somerset Drive, Belmont, CA 94002 (US). GODDARD, Audrey [CA/US]; 110 Congo Street, San Francisco, CA 94131 (US). GURNEY, Austin [US/US]; 1 Debbie Lane, Belmont, CA 94002 (US). HILLAN, Kenneth [GB/US]; 64 Seward, San Francisco, CA 94114 (US). LAWRENCE, David, A. [US/US]; 1659 12th Avenue, San Francisco, CA 94122 (US). ROY, Margaret [US/US]; 2960 Webster Street #4, San Francisco, CA 94123 (US). WOOD, William, I. [US/US]; 1900 Tarrytown Street, San Mateo, CA 94402 (US).

(74) Agents: DREGER, Ginger et al.; Genentech, Inc, 1 DNA Way, South San Francisco, CA 94080–4990 (US).

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(54) Title: GENES AMPLIFIED IN TUMOURS, ANTIBODIES AGAINST THE PROTEINS CODED THEREBY, AND THEIR USE IN DIAGNOSIS AND TREATMENT OF CANCER

(57) Abstract

The invention concerns compositions and methods for the diagnosis and treatment of neoplastic cell growth and proliferation in mammals, including humans. The invention is based on the identification of genes that are amplified in the genome of tumor cells. Such gene amplification is expected to be associated with the overexpression of the gene product and contribute to tumorigenesis. According, the proteins encoded by the amplified genes are believed to be useful targets for the diagnosis and/or treatment (including prevention) of certain cancers, and may act of predictors of the prognosis of tumor treatment.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/18824

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K16/18 C07K16/22 C07K16/28 G01N33/53
C12Q1/68 C07K14/50 C07K14/47 C07K14/705 C07K14/475

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 39508 A (HUMAN GENOME SCIENCES INC ;GREENE JOHN M (US); GRUBER JOACHIM R (U) 12 December 1996 (1996-12-12) page 22, paragraph 4 - page 23, paragraph 3; claims 10,14,18,20 page 24, paragraph 3 ---	1,4-6, 18-22
A	WO 96 38509 A (NOCOPI INT INC) 5 December 1996 (1996-12-05) page 30, line 25 - line 31 ---	1,4-6
A	WO 94 00603 A (UNIV PRINCETON ;SLOAN KETTERING INST CANCER (US); LEVINE ARNOLD J) 6 January 1994 (1994-01-06) the whole document ---	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 April 1999	05.08.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Smalt, R

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna··nal Application No
PCT/US 98/18824

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 20238 A (UNIV JOHNS HOPKINS) 14 October 1993 (1993-10-14) the whole document -----	

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In ational application No.
PCT/US 98/ 18824

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 18-20, in as far as they relate to use in vivo, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet.

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Claims 1-25.

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-25

Antibodies binding to PRO187 and compositions comprising it, its use in determining the presence of PRO187 in a sample, method of diagnosing a tumor comprising determining the presence of PRO187 in a test sample, method of inhibiting the growth of a tumour-cell in vitro using said antibody, diagnostic ore therapeutic kit containing said antibody, and a method to identify compounds capable of inhibiting the expression or activity of PRO187.

2. Claims: 1-25

Antibodies binding to PRO533 and compositions comprising it, its use in determining the presence of PRO533 in a sample, method of diagnosing a tumor comprising determining the presence of PRO533 in a test sample, method of inhibiting the growth of a tumour-cell in vitro using said antibody, diagnostic ore therapeutic kit containing said antibody, and a method to identify compounds capable of inhibiting the expression or activity of PRO533.

3. Claims: 1-25

Antibodies binding to PRO214 and compositions comprising it, its use in determining the presence of PRO214 in a sample, method of diagnosing a tumor comprising determining the presence of PRO214 in a test sample, method of inhibiting the growth of a tumour-cell in vitro using said antibody, diagnostic ore therapeutic kit containing said antibody, and a method to identify compounds capable of inhibiting the expression or activity of PRO214.

4. Claims: 1-25

Antibodies binding to PRO240 and compositions comprising it, its use in determining the presence of PRO240 in a sample, method of diagnosing a tumor comprising determining the presence of PRO240 in a test sample, method of inhibiting the growth of a tumour-cell in vitro using said antibody, diagnostic ore therapeutic kit containing said antibody, and a method to identify compounds capable of inhibiting the expression or activity of PRO240.

5. Claims: 1-25

Antibodies binding to PRO211 and compositions comprising it, its use in determining the presence of PRO211 in a sample, method of diagnosing a tumor comprising determining the presence of PRO211 in a test sample, method of inhibiting the growth of a tumour-cell in vitro using said antibody,

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

diagnostic ore therapeutic kit containing said antibody, and a method to identify compounds capable of inhibiting the expression or activity of PRO211.

6. Claims: 1-25

Antibodies binding to PRO230 and compositions comprising it, its use in determining the presence of PRO230 in a sample, method of diagnosing a tumor comprising determining the presence of PRO230 in a test sample, method of inhibiting the growth of a tumour-cell in vitro using said antibody, diagnostic ore therapeutic kit containing said antibody, and a method to identify compounds capable of inhibiting the expression or activity of PRO230.

7. Claims: 1-25

Antibodies binding to PRO261 and compositions comprising it, its use in determining the presence of PRO261 in a sample, method of diagnosing a tumor comprising determining the presence of PRO261 in a test sample, method of inhibiting the growth of a tumour-cell in vitro using said antibody, diagnostic ore therapeutic kit containing said antibody, and a method to identify compounds capable of inhibiting the expression or activity of PRO261.

8. Claims: 1-25

Antibodies binding to PRO246 and compositions comprising it, its use in determining the presence of PRO246 in a sample, method of diagnosing a tumor comprising determining the presence of PRO246 in a test sample, method of inhibiting the growth of a tumour-cell in vitro using said antibody, diagnostic ore therapeutic kit containing said antibody, and a method to identify compounds capable of inhibiting the expression or activity of PRO246.

9. Claims: 1-25

Antibodies binding to EBAF-2 and compositions comprising it, its use in determining the presence of EBAF-2 in a sample, method of diagnosing a tumor comprising determining the presence of EBAF-2 in a test sample, method of inhibiting the growth of a tumour-cell in vitro using said antibody, diagnostic ore therapeutic kit containing said antibody, and a method to identify compounds capable of inhibiting the expression or activity of EBAF-2.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/18824

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